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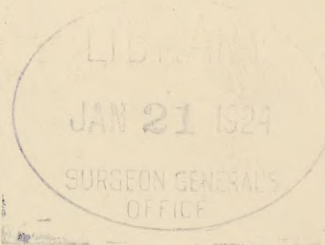
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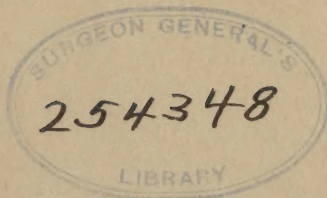
A LABORATORY COURSE IN PHYSIOLOGY

BY

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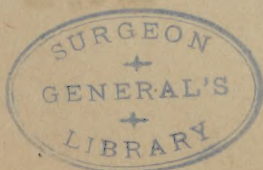
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PREFACE

To the student of biology and the student of that part of applied biology included in medicine, the laboratory method of learning the functions of the tissues and organs of the animal body has demonstrated its superiority over other methods, not only in impressing vividly the facts of observation, but also in demanding a discipline which furnishes to the student power to go forth with assurance in independent search.

That the student may learn the facts of the science important for his further progress, experiments must be selected which have for that purpose the most significance. That the student may receive the greatest benefit from the discipline of his study, it is essential that he be placed so far as possible on his own resources. He should be required, under supervision, to make and record his own observations, and draw his own conclusions, and should be urged at every step to suggest further observation or experiment to test those conclusions. A student who has conscientiously applied his abilities under such requirements possesses at the end a first-hand acquaintance with the subject-matter of physiology, and has formed besides a very valuable set of habits — the habits of careful observation, of concise and accurate statement of things observed, and of exact inference from these observations. He may also have been stimulated to exercise his imagination in the planning of further experimentation to discover new knowledge.

That the purposes of laboratory instruction may be served, the attempt has been made to arrange the experiments in this book, in both the larger and smaller divisions of the subject, so as to render answers in an orderly manner to the following questions: —

First. Of what activity is an organ or tissue capable?

Second. How do certain factors condition or modify that activity?

An attempt has been made also to arrange the experiments so that facts with widest bearing are observed first. Such a plan permits a statement of subsequent experiments in a manner which will call forth the greatest use of the student's intelligence — he is required repeatedly to rely on his previous observations.

Much importance of the facts of observation lies in their relations to one another, that is, in the generalizations which may be drawn after comparing them. To stimulate students to regard thoughtfully what they see and to note the relation of present phenomena to those already observed, questions and suggestions have been found valuable. They tend to prevent the laboratory exercise from degenerating into an automatic following of directions in the book, and an automatic recording of what the book states will occur. The student should consider carefully any questions given in the text or suggested during the course of experimentation.

It is important for the student to realize that in performing his experiments he is following the ancient way which led to knowledge — in short, that he is learning the methods of progress by repeating the procedures of the original discoverers. In order to make this relation clear, references are frequently given to the first publication of the method and the results. In some instances, also,

references are made to related literature, which will permit the student to become better acquainted with the significance of his work, in case his interest is aroused.

The selection of experiments has been made on the assumption that students will have additional practical experience in biological chemistry.

To save the student's time and to afford him at the end of the course a concise and convenient account of his laboratory experiences, the descriptions of experiments have been printed only on the left-hand pages in order that the notes may be written opposite on the right-hand pages. When a graphic record is made it should be preserved with the notes on observations. The paper bearing the record should be carefully trimmed, then pasted in the book and protected by tissue-paper from disfigurement by rubbing. From the beginning the student should plan to record his results in an orderly manner, giving the conditions of the experiment, the observations made, and the inference, if an inference may properly be drawn. The notes should be written as soon as the experiment is finished. They should be brief and clear. Diagrams often serve better than verbal descriptions.

The directions for experimentation here presented are the result of numerous corrections and alterations, based on experience with large classes. I wish to express my gratitude to Dr. E. G. Martin, Dr. E. B. Meigs, and Dr. R. G. Hoskins for many valuable suggestions in the earlier development of the "Course," and especially to Dr. C. K. Drinker and Dr. A. C. Redfield for important contributions to later editions.

PREFACE TO THE FOURTH EDITION

To lessen the pressure on the time of medical students, the course in physiology at the Harvard Medical School was reduced during the past year to about 230 hours, i.e., it was shortened about one-third. At the same time, the important recent development in our knowledge of metabolism and blood gases has led to the inclusion of new experiments requiring expensive apparatus. In order to adjust the laboratory course to these conditions, the class works as a whole, for three weeks, on the experiments described in the first 32 pages. Because of the wide significance of the "all-or-none" law, the fundamental observations of Bowditch have been transferred to this part of the course. After the first three weeks the class is divided into four sections, and the laboratory work is likewise divided into four parts — the nervous system and the receptors, the circulation, body fluids, and metabolism and respiration. The sections perform the experiments in these parts of the course in turn, with explanatory talks and conferences accompanying each step. Meanwhile, demonstrations and lectures are given on parts of physiology not thus taken up in the student laboratories. The reduction in the time available for laboratory work has required the elimination of many experiments. Those omitted from the course at the Harvard Medical School are designated by an asterisk. They have been retained in the present edition for students who may wish to perform them, or for teachers who may wish to include them in laboratory instruction.

I wish to express my thanks to Dr. K. S. Drinker, Dr. C. K. Drinker, Dr. A. Forbes, Dr. F. R. Griffith, Dr. A. C. Redfield, and Mr. H. O. Veach for suggestions and contributions to the present edition.

A LABORATORY COURSE IN
PHYSIOLOGY

IRRITABILITY, STIMULATION, AND METHODS OF RECORDING

THE most general property of living structures that can be readily demonstrated is irritability — the property of responding in a characteristic manner to the action of external agents serving as stimuli. The response varies greatly; it may be growth, it may be movement, or secretion, or increased production of heat, or it may be the diminution or cessation of these activities. Likewise the external agents which may affect the activities of living organisms are very diverse, — mechanical contact, the vibrations of air, chemical agents, the rise or fall of temperature, and electricity and light are all stimuli capable of conditioning, under favoring circumstances, the discharge of energy from the organism.

The greatest amount of bodily energy is liberated as mechanical work and as heat incident to the performance of mechanical work. The active agents concerned in doing external work in the higher organisms are neuro-muscular structures. The movements of the body, whereby the environment is changed, are performed by muscles. But the muscles are controlled and their activity directed by means of nerves. And the nerves are, in turn, acted upon by stimuli — agents external to the nervous system — impressing the nerves in various sense organs. It is thus through the nervous system that its sense organs, on the one side, serve to adapt the actions of its end organs, the muscles, on the other side, to the character of the surroundings, and to modify those surroundings to suit the needs of the organism.

Muscle and nerve have been called, therefore, the “master tissues.” By them the organism acts to preserve itself. The other tissues and systems of organs may be regarded as subservient to these master tissues. In order that the master tissues may perform their functions, the circulatory system, the activity of which depends on a special form of muscle and nerve, maintains through the flow of blood and lymph a supply of energy in the form of food and a supply of oxygen for the release of that energy. And the circulatory system likewise removes the waste, and distributes throughout the body the heat incident to muscular contraction. To supply to the blood and lymph the necessary food, properly prepared, as well as the necessary oxygen, secretion, digestion, and absorption occur, and also respiration; and to rid the blood of its waste the organs of excretion play their part. That these internal exchanges may be properly regulated, that excess of absorbed food may not be lost, and that undestroyed waste may not disturb the normal functionings, the processes of metabolism make good the wear and tear, store the abundance till time of need, and burn the waste to the end-products that are discharged by lungs, kidneys, and skin.

Because muscle and nerve are the master tissues, because their activities are most generally distributed in skeletal movement, in the organs for circulation, digestion, respiration, and the production of animal heat, and because the study of the activities of muscle and

nerve involves a training in general methods and offers an introduction to conceptions useful in other divisions of physiology, they are studied first.

Pithing a Frog.—Wrap a frog in a cloth and hold him head upward in the left hand. Press the front of the head downward with the left index finger, thus making a bend at the occipito-vertebral junction. Push the point of a pithing needle directly down into the depression between the skull and the first vertebra, and move the point quickly from side to side, thus cutting across the medulla. Push the needle forward into the skull cavity to destroy the brain, and downward into the vertebral canal to destroy the spinal cord. Note what happens at each stage of this operation. Is there any difference in the feel of the frog after the spinal cord is pithed? The significance of these changes will be considered later. The frog as an organism is now dead, but surviving parts will still exhibit their functions.

The Nerve-muscle Preparation.—After pithing a frog, sever with heavy scissors the spinal column close behind the fore limbs. Seize the lower portion of the column with heavy forceps. Cut downward through the body wall on either side, and remove the front part of the body together with the viscera. With heavy scissors split the vertebrae and pelvis, making two similar leg preparations. As the skin is a good preservative of the underlying tissues, leave one leg in its natural covering until needed. Strip the skin from the other leg. At once throw the skin into the waste basin, for on the outer surface is a secretion injurious to the internal tissues. Lay the bare muscles on a clean dry plate.

The most convenient muscle to study is the gastrocnemius. The sciatic nerve, which supplies the gastrocnemius, lies on the dorsal surface of the thigh between the ileofibularis and the semimembranosus. Carefully separate these muscles, and expose the nerve up to the pelvis. Divide the pyriformis. With care for the underlying nerves cut the coccygeo-iliac muscle along its attachment to the os coccygis or urostyle, the long slender bone at the end of the spinal column. With forceps pick up the half vertebrae and thus lift through the opening in the muscle the three cords of the sciatic nerve. Holding up the nerve without stretching it, cut its small branches and isolate it down to the lower end of the femur. Lay the isolated nerve on the gastrocnemius muscle. Remove from the lower two thirds of the femur its muscular attachments, but be careful not to injure the sciatic nerve. Cut off the uncovered part of the femur.

If the femur is now fixed in a flat-jawed clamp, with leg and foot upward, the contraction of the long anterior tibial muscle will cause the leg to be strongly extended. For demonstration purposes this preparation is sufficient. It will be referred

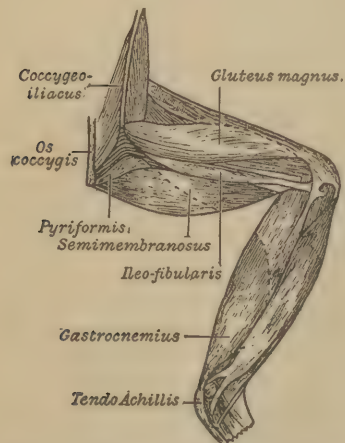


FIG. 1.—Muscles of frog's thigh and leg, dorsal aspect (after Gaupp).

to hereafter as the "demonstration preparation."

THE CLASSES OF STIMULI

In studying the functions of tissues and organs it is first necessary to become acquainted with the agencies which may be used to cause these irritable structures to manifest their peculiar activities. The nerve-muscle preparation will serve to show the peculiarities of most of these agencies, and their relative serviceableness. The experiments on mechanical and thermal stimuli must be performed as soon as possible after removal of the preparation from the body. The reason appears later.

1. Mechanical Stimuli

Fasten the femur in a femur clamp which is supported on an iron stand at the height of a beaker. Lay the nerve of the preparation on the bottom of the inverted beaker. Strike the nerve lightly near its central (vertebral) end with a pithing needle. What occurs?

Pinch the nerve sharply with small forceps. Describe the change in the muscle. Is there any visible change in the nerve?

Stimulate the nerve central to the point at which it has already been pinched. State one of the defects of mechanical stimulation.

Give an example of mechanical stimulation of a nerve in the body.

2. Thermal Stimuli

Touch the nerve with the pointed end of a glass rod so gently that there is no response. Heat the glass rod cautiously and again touch the nerve as gently as before. Note the response.

Stimulate central to the point where the hot glass rod was applied. State a defect of the use of heat as a means of stimulation.

3. Physico-chemical Stimuli

Drying. — Let the nerve of the preparation become gradually dry. Note the activities of the muscle. The student now understands the need for immediate observation of an excised nerve unless means are used to prevent drying. Test the function of the nerve by a method already used. If this function becomes lost, apply to the nerve 0.7 per cent sodium chloride by means of a camel's hair brush. Repeat this procedure several times.

Does the function of the nerve return?

***Strong Saline Solutions.** — Remove the ileofibularis muscle of the frog's thigh and suspend it for ten minutes half-immersed in a strong (15 per cent) solution of sodium chloride. Note what occurs immediately on immersion and in the course of the ten minutes.

***Distilled Water.** — Remove a sartorius muscle from the frog. To do this seize in small forceps the sartorius tendon at the tibial insertion and with sharp small scissors cut it from its attachment to the bone. Lift the freed end gently and separate the muscle up to the origin by snipping the fascia on either side. Sever the muscle at the origin and suspend it half-immersed in distilled water. Note any movements in the muscle; note any change of length or volume. Does it change color?

The experiments on the effects of drying prove that loss of water may bring about internal changes in a nerve which result in spasmodic activity of the muscle to which it is attached. The tissues of the body are composed of approximately sixty-five per cent water.

In this water are dissolved salts and colloids. These solutions are held in minute spaces by the structural membranes of the body. Through these membranes fluids may pass in and out. Thus to a large degree may be explained the effects of strong salt solution and of distilled water already observed. But muscles in the body do not twitch as they are observed to twitch when in strong salt solution or in distilled water. The moving fluids of the body — blood and lymph — must be “normal” to the fluids in the muscle in order to prevent unnatural interchange through separating membranes, with such results as have been demonstrated above. A “normal” fluid, a fluid having the same salts as the blood, in like concentration, may be used to replace the blood in part after haemorrhage. It may also be used to keep surviving tissues or organs in a natural state. It is important, therefore, to know the osmotic pressure of the blood.

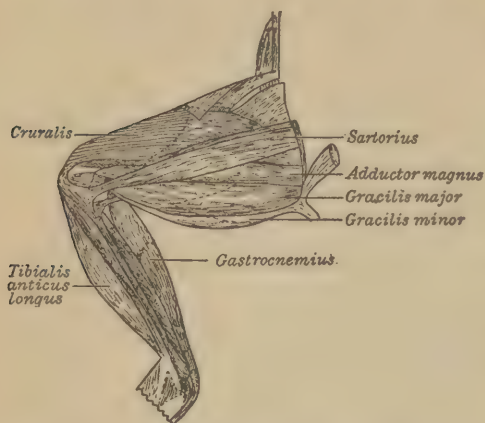


FIG. 2. — Muscles of frog's thigh and leg, ventral aspect (after Gaupp).

The osmotic pressure of a solution can be determined in different ways, *e. g.*, by finding to what degree the freezing point is lowered, or by noting what occurs when a semipermeable membrane (a membrane permitting water to pass but restraining dissolved substances) separates the solution from another known solution.

(**Demonstration. — Osmotic Pressure.* — Into a parchment-paper bag is fastened a rubber cork provided with a hole into which a small glass tube will fit snugly. The bag is filled with a 30 per cent sugar solution. Into the rubber cork is inserted a long piece of glass tubing. The glass tubing is now supported so that the bag rests in a beaker filled with water. The fluid in the bag soon begins to increase in amount and rise in the glass tube. After this process has continued for some time and the sugar solution has risen two or three feet in the tube, the beaker of water is replaced by a beaker containing 60 per cent sugar solution. The fluid in the tube now begins to fall and falls continuously.)

These observations show how a fluid moves when, on opposite sides of a membrane, the numbers of particles in solution are different. If the numbers were the same on the two sides, there would be a state of equilibrium, the two sides would be balanced, and the fluid, if it passed the partition, would pass as much in one direction as in the other.)

One of the earliest methods of determining osmotic pressure was that used by botanists in studying the effects of various concentrations of salts on plant cells. Plant cells possess a relatively rigid cell membrane lined within by a layer of protoplasm, the primordial sheath, which encloses the fluids of the cell. The primordial sheath is to a certain extent a natural semipermeable membrane, for it prevents the escape of the salts and sugar of the cell sap, and yet permits free entrance of water. If the cell is placed in water or in a solution of less osmotic pressure than that of the cell sap, water enters the cell and the cell becomes turgid. The fluid outside the cell is then said to be *hypotonic* to that in the cell. If, on the other hand, the cell is bathed by a solution of greater osmotic pressure than that of the cell sap, water passes out and the primordial sheath shrinks away from the cell membrane. This is plasmolysis. A solution causing plasmolysis is *hypertonic* to the fluid contents of the cell.

Osmotic Properties of Cells. — 1. *Resistance of Red Corpuscles to Hypotonic Solutions.* Prepare a series of nine small test tubes each containing about 2 c.c. of sodium chloride solution, ranging in concentration from 0.5 per cent to 0.18 per cent by steps of 0.04 per cent. Dilutions may be made conveniently from a stock of 0.5 per cent NaCl by counting out the drops delivered from a pipette (to make a total of 50 drops, each successive tube to receive 4 drops more of water and 4 drops less of 0.5 per cent NaCl) or they may be measured with a graduated pipette, each successive tube to receive 0.16 c.c. more of water and 0.16 less of the NaCl solution in a total of 2.0 c.c.

Prick the finger and obtain several drops of blood in a paraffined watch glass (which will retard clotting for several minutes). Suck up 20 cu. mm. with the haemoglobinometer pipette and add this amount of blood to each tube. Care must be taken not to allow time for the blood to clot in the pipette which is then very difficult to clean. *Rinse the pipette at once with distilled water and dry with alcohol and ether.*

Set the tubes aside for several hours. Then observe: —

1. In which tubes the salt solution is colored with haemoglobin — the upper limit of fragility.
2. In which the corpuscles have collected at the bottom and are still intact — the lower limit of fragility.

Estimate the molecular concentration of the solutions at these limits. (Molecular weight of NaCl = 58.5.)

II. *Salts which Penetrate the Corpuscles do not Prevent Haemolysis.*

Prepare tubes containing 2 c.c. of (a) 0.9 per cent NaCl (0.154 molar), (b) 1.8 per cent urea (0.3 molar) and (c) 0.9 gr. NaCl + 1.8 gr. urea in 100 c.c. of water.

Add 20 cu. mm. of blood, as before.

In which tubes does haemolysis occur?

Is the effect of the urea solution due to its osmotic or its chemical properties?

III. *The Limits of Fragility are Related to the Osmotic Properties of the Salt Solution, rather than to its Molecular Concentration.*

Prepare nine tubes containing glucose solutions ranging from 0.15 molecular to 0.054 molecular by steps of 0.012 molecular. (Each step is equivalent to 4 drops in 50 or 0.16 c.c. in 2.0 c.c.)

Determine the upper and lower limits of fragility in the glucose solutions.

Isotonic solutions are solutions of different salts having the same osmotic pressure. What molecular concentrations of glucose and of NaCl are isotonic as judged by the limits of fragility? The isotonic coefficient of NaCl is given by

$$\frac{\text{Molecular concentration of glucose}}{\text{Molecular concentration of NaCl}}$$

when both are isotonic. Calculate it. Explain why NaCl is isotonic with glucose at a lower molecular concentration.

The proportion of molecules of NaCl split into ions may be calculated from the isotonic coefficient as follows. If the isotonic coefficient be considered as measuring the total number of molecules and ions present as the result of solution of one unit quantity of sodium chloride, then the total number of particles or the isotonic coefficient (i) = [number molecules dissociated (a) \times number ions from each molecule (K)] + [total number of molecules (1) — number molecules dissociated (a)]; or $i = aK + 1 - a$, or $1 + a(K - 1)$; for $\text{NaCl} \rightleftharpoons \text{Na}^+ + \text{Cl}^-$ and $K = 2$.

Calculate the degree of dissociation (a) for NaCl.

4. *Swelling and Shrinking of Corpuscles in Relation to Osmotic Pressure.*

Prepare a series of dilutions of NaCl ranging from 0.4 per cent to 2.0 per cent. Place 0.5 c.c. of each in a watch glass and add a few cubic millimeters of blood to each. Transfer a drop of the mixtures to a microscope slide and cover it with a cover glass. Wait 15 minutes and then measure the diameter of about ten corpuscles from each mixture with the ocular micrometer scale of the microscope. Plot the average diameter of the corpuscles against the concentration of the solution. At what concentrations does "crenation" appear? To what is it due?

Place a drop of *blood* on a slide and cover it. Determine the diameter of the corpuscles while suspended in plasma. At what concentration of NaCl are the corpuscles of normal size?

This experiment may be repeated with glucose solutions ranging from 0.125 to 0.60 molecular and the isotonic coefficients and the degree of dissociation recalculated.

4. Electrical Stimuli

The observations already made on mechanical, thermal, and chemical agents as means of bringing tissues into activity show that these agents have serious defects. They are likely to injure or destroy the tissues, and the accurate control of the intensity and duration of their application is difficult. The frequent necessity of continuous stimulation, or of repeated stimulation of uniform intensity, without injury to the tissues, makes highly desirable an agent capable of satisfying these requirements. Such an agent is provided in the induced electric current.

The student will recall from his previous study of physics that if the current in a wire is made or broken, or altered in strength, a current is induced in a second wire placed near the first. The student will also recall that while a current runs in a wire the wire is surrounded by a whirl of magnetic lines of which the wire is the axis, and that the inductance of a current in a neighboring wire is due to the cutting of that wire by these lines of magnetic force. The strength of the induced current depends on several factors: it varies directly with the current change in the first wire, and with the rate of change of the inducing current; it varies inversely with the distance between the two wires; it varies with the angle between the two wires, being greatest when the wires are parallel to each other and entirely absent when they are at right angles to each other. These facts are utilized in making an inductorium.

The Construction and Use of the Inductorium. — The inductorium consists of two coils of wire: a primary coil of a relatively small number of turns of fairly stout wire, and a secondary coil of many more turns of much finer wire. The strength of current induced in the secondary coil can be increased by placing inside the primary coil a core consisting of a bundle of soft iron rods. The iron rods, by their great magnetic permeability, increase the number of lines of magnetic induction which pass through the core from end to end and return outside through the air. By the formation of these lines the core itself becomes magnetized, and when they disappear it becomes demagnetized. The secondary coil is wound on a large hollow spool which can be moved over the primary or withdrawn and turned at various angles with the axis of the primary. Thus the induced current may be caused to vary from zero, when the secondary is withdrawn and turned at right angles to the primary, up to the maximum when the secondary covers the primary.

As already noted the current is induced in the secondary coil only when there is an alteration of the strength of current in the primary; when that change is instantaneous, as in the breaking of the primary circuit, the induced current is also instantaneous. The induced current produced by the making of the primary current runs opposite to the direction of the primary; thus, if the current runs clockwise in the turns of the primary coil, the induced current runs anti-clockwise in the turns of the secondary. On suddenly breaking the primary current the induced current is in the same direction as was the primary before the break.

It is of importance to note that not only does the current in the primary circuit react on the wires of the secondary coil, but each turn of the primary can induce currents in each neighboring turn. The formation of a current in one turn will thus induce a current in the neighboring turns and as the current induced in the neighboring turns is opposite in direction to the main current, this self-induced current tends to retard the development of the main current. The duration of the self-induced current, however, is short and the main current, therefore, soon rises

to full strength. On breaking the primary circuit a similar self-induced current is provoked which, however, is very brief and prolongs slightly, in the spark, the duration of the main primary current.

Since the currents induced in the secondary coil occur during the change of intensity of the primary, there is evidently a current in the secondary only during the formation of the primary current and again at the instant that the primary is broken. And since the rise of the primary current to full strength takes longer than the fall to zero, the induced current is less intense at the make than at the break. The make shock is therefore less effective than the break.

It is frequently desirable to have a series of rapidly repeated stimulations. Instead of making and breaking the primary current by hand it is possible to produce automatically a series of rapid stimulations by means of a mechanical interrupter on the inductorium. Examine the wires of the apparatus and find the connections which cause the current to pass through the screw the point of which is in contact with a flat spring. The flat spring is connected with the primary coil. When the circuit is properly made the current passes through the screw to the spring and thence through the primary coil, magnetizing the iron core. The magnetized core pulls the iron disc fastened to the spring and thus breaks the circuit between the spring and the screw. As the current is no longer running the core becomes demagnetized, the iron disc is no longer pulled, and the spring flies back against the screw. The circuit is again made and the process again repeated. Thus the primary circuit is rapidly made and broken, and with each make and break a current is induced in the secondary coil. The quickly alternating induced currents form the so-called "tetanizing current."

Induced currents have so small an amperage and so brief a duration that polarization (see p. 12) is very slight, and when make and break currents follow one another alternately the reversal of direction almost wholly obviates polarization. For these reasons the use of platinum electrodes with induced currents is justified.

The Simple Key. — The simple key is used to complete or to interrupt a circuit by making or breaking contacts, commonly platinum. Differences in the rate of making or breaking the primary current, slipping of the contacts, and possibly other conditions, are likely to cause variations in the currents induced in the secondary coil. A copper wire dipping into clean mercury serves as a very satisfactory key.

Make and Break Induced Currents as Stimuli. — Make a nerve-muscle preparation for demonstration. Arrange the inductorium to produce single shocks. Attach to the secondary coil the platinum electrodes. Move the secondary away from the primary and turn it at right angles to the axis of the primary. Touch the electrodes to the nerve. Make and break the primary circuit. What occurs? Explain the result.

Turn the secondary gradually toward the axis of the primary and make and break the primary circuit at each step. When the muscle contracts at the make and at the break of the primary circuit, note whether there is any contraction while the primary current is flowing. If not, why not?

Between the posts of the secondary coil is a metal bar which can be raised and lowered. Lower the bar so as to connect the two posts. Now make the primary current. Does the muscle contract?

Raise the bar and break the primary current. Does the muscle contract?

Make the primary current. Does the muscle contract?

Lower the bar and break the primary current. Does the muscle contract?

While the primary current is evenly running, make and break the secondary circuit through the bar. Explain the result.

Repeat, using the tongue to test the effectiveness of the stimuli.

The electric current when offered two paths to the same end divides inversely as the resistance. Since the resistance of the nerve is enormously greater than the resistance of the bar, practically none of the current passes through the nerve when the bar connects the two posts. The bar, therefore, is an effective short-circuiting key and can be used whenever it is desirable to eliminate either the make or the break induced current.

THE GRAPHIC RECORD

Sometimes physiological processes are so rapid that it is impossible for the eye to follow them, sometimes they are so slight that they must be magnified in order that they may be studied. In almost all cases it is desirable to have permanent objective records of what has taken place. For these various reasons ingenious devices have been invented whereby all manner of physiological changes are recorded. The most generally applicable of the devices for securing an objective record are the kymograph and the writing lever.

The Kymograph. — The essential feature of a kymograph is a cylindrical drum which is rotated about its axis at a fairly uniform rate by a mechanism with an adjustable speed (Ludwig, Arch. f. Anat. u. Physiol., 1847, p. 242, tables x-xiv). Study the method by which the speed is controlled. If the mechanism is impelled by a spring, take care not to wind the spring very tight, for the coils may in that case bind on one another and thus fail to operate. The use of the kymograph depends on its presenting a moving surface on which physiological processes may record themselves. The surface is usually glazed paper, coated with a film of smoke; and the record is made by a writing point rubbing off, as it moves, a trace in the film of smoke.

The glazed paper to be applied to the drum is provided in sheets wider than the length of the drum and slightly longer than its circumference; each sheet is gummed along one end. To apply the paper lay it on the table, glazed surface downward, and gummed end distant. Place the drum across the middle of the paper, draw the near end of the paper snugly around the drum's surface and hold it tightly in place with the thumbs. Roll the drum forward, and raise the gummed end of the paper between the thumbs and forefingers. The edges of the paper should be continuous at either side. With the paper pulled snugly around the drum, moisten the gummed edge and press it in place. Unless care has been taken to fasten the paper tightly about the drum, it will slip down when the drum axis is vertical. *Do not trim off the projecting paper at this time.*

The drum is now lifted by the rod passing through its axis and the paper is smoked. The gas flame used in smoking should be a moderate, steady flame, neither so high as to flare, nor so low as to flicker. Support the rod by the first two fingers of each hand with the tips of the fingers pointed downward toward the body. Let the rod roll down the fingers and be caught each time before it is in danger of rolling off. Thus the drum is given a uniform rate of revolution. The rate should not be rapid. Lower the drum into the upper edge of the flame and rotate it slowly and evenly until the glazed surface is covered with a chocolate-colored film of smoke.

The paper should now be trimmed from the edges; to do this hold the rod in one hand and with the other hand press a knife against the edge of the drum. The knife and the drum-edge act like the two parts of a pair of shears and cut the paper between them. Immediately put the separated rings of paper in the waste basket.

After a record has been written the paper is removed. In doing this hold the rod in one hand with the thumb pressed on the corner of the overlap. With a sharp knife cut through the overlap along the gummed edge. Still holding the paper in place with the thumb allow the loosened end to fall away from the drum. The paper may then be held by the unsmoked overlap.

The record must now be made permanent. This is done by passing it through a solution of shellac or resin. Hold the unsmoked overlap in the right hand and raise the other end of the paper until the bend is in the unsmoked portion. Dip the bended part in the pan of the fixative. Now raise the unsmoked end and lower the other end at the same rate, passing the entire sheet through the solution. If the sheet is now hung up to dry the gum hardens and fixes the smoke permanently in place.

The Writing Lever. — The purpose of the lever is to write a magnified record of the movements of the structure to which it is attached. In order to avoid the inertia of position and also the inertia of motion, it is desirable that the lever be as light as possible, consistent with rigidity. In practice a straw is found to fulfil these conditions satisfactorily. In place of a straw, however, a thin strip of aluminum may be employed.

The straw should not write directly on the smoked surface. A writing point is attached to the lever for this purpose. The writing point is made of a piece of parchment paper about 7 mm. wide and about 2 cm. long, brought to a sharp angle at one end by cutting off the corners. The broadness of the strip of parchment paper prevents it from being twisted as the point moves on the smoked surface. In preparing the straw for the writing point sharpen the small end by a long oblique cut with a knife. Place some colophonium wax in the hollow of the cut tip. Melt the wax by means of a hot wire, and press the broad part of the paper into the melted wax so that the writing point projects freely about 1.5 cm.

To attach the straw to the muscle lever, place its uncut end in the ring of a double hook, fit it over the tapering tube of the muscle lever, and hold it in place by pushing the double hook as far as possible over the straw as it spreads on the tapering surface. *The axis of the muscle lever should always be horizontal* and the surface of the writing point should always be vertical. Why? It is well to have the cut side of the straw facing the drum and the projecting portion of the writing point bent slightly toward the drum. This bend makes a weak spring which serves to keep

the writing point on the smoked surface, in spite of the fact that the surface curves away from the plane of movement as the lever rises.

N. B. Since the drum rotates from right to left, *always set the recording apparatus pointing toward the left.* The writing lever, with its axis horizontal, should be clamped level with the lower border of the drum when the drum is raised on its axis as high as possible. This precaution insures the possibility of using all of the smoked surface without changing the apparatus.

Always have ready the apparatus for recording and stimulating before preparing the tissues to be studied. As already learned, they are likely to deteriorate rapidly on standing outside the body.

SOME GENERAL PHENOMENA OF STIMULATION

The Threshold Value of a Stimulus.—To prepare the gastrocnemius muscle (without its nerve) for attachment to a lever, proceed as directed on page 4, but separate the tendon from the bone as far as possible below the ankle joint. Grasp the tendon and pull the gastrocnemius free up to its attachment to the femur. Cut through the tibia just below the knee joint. Fasten in a heavy clamp the femur supporting the gastrocnemius muscle. Insert into the tendon of the muscle the pointed end of an S-shaped pin hook. Attach the pin hook to the writing lever already described. To complete the circuit use a fine copper wire to connect the pin hook with the binding post on the muscle lever. The wire should not be taut. Load the muscle with a 10-gram weight placed in a scale pan hanging below the muscle. Do not support the weight by the screw adjusting the lever, the "after-loading screw." Connect the secondary coil of an inductorium to the binding post on the muscle clamp and to the binding post on the lever. Attach a dry cell through a simple key to the primary coil so as to produce single shocks.

Let the writing point draw an abscissa on the smoked paper as the drum is turned by hand. With the drum at rest stimulate the muscle with the weakest possible break induction current. There should be no response. Gradually increase the strength of the secondary current and stimulate with break shocks as the strength increases. A point will be reached at which the muscle makes the slightest contraction. This weakest stimulus causing any contraction at all is the minimal stimulus. And the point at which an increasing strength of stimulus passes from a region of ineffectiveness into a region of effectiveness is called the "threshold value" of that stimulus.

The Summation of Inadequate Single Stimuli.—Arrange the secondary coil to send a just subminimal break shock through the muscle. After the muscle has rested for a moment stimulate it by rapidly making and breaking the current.

If one looks at a bright light for a moment and then shuts the eyes, the sensation of light persists after the stimulus ceases to affect the eye. Does this persistence of the excited state suggest an explanation for the effects following the repeated application of inadequate stimuli? State fully your explanation of the effects.

Submaximal and Maximal Stimuli.—Arrange the secondary coil to send through the muscle a minimal break stimulus. Record this slightest contraction on the stationary drum. Turn the drum a half centimetre, slightly increase the strength of the induced current, and stimulate with a make shock. If there is no response, stimulate a moment later with a break shock. The make and break shocks should be produced by as uniform a manipulation of the key as possible. Continue the record, increasing slightly the strength of the induced current before each pair of make and break stimuli, turning the drum by hand a half centimetre after each recorded contraction, and taking particular care to *avoid summation of excitations.*

A point will be reached in the gradual increase of the strength of the induced currents at which the highest contraction of the muscle stimulated with a break shock will occur. The stimulus just producing this highest contraction is a maximal stimulus. The strength of the induced current may continue to increase, but the muscular response does not. Any stimulus less than that producing a maximal contraction is known as a submaximal stimulus. As the induced currents are increased in strength, do the minimal and maximal break stimuli occur at the same points at which the minimal and maximal make stimuli occur?

What do you conclude as to the relative efficiency of make and break stimuli?

To what physical characteristics of make and break stimuli already discussed may this difference in physiological effect be ascribed?

Lay the muscle in a pool of isotonic salt solution on a glass plate. Place in the solution, at a distance of one or two centimetres from the muscle, the points of the platinum electrodes. Cause a maximum induced current to flow through the electrodes. The muscle will contract even though the electrodes are not in contact with it. How may this result be explained?

It is evidently unnecessary in stimulating an irritable tissue to use a stimulus stronger than the maximal stimulus; it is furthermore undesirable, for strong stimulations are likely to injure the tissues and thus disturb physiological states and are likely also to rouse to activity other tissues than those being studied. *The weakest stimulus which will produce the desired effect should always be used.*

It is highly important to learn proper methods at the start. When you have completed the foregoing experiments *request the demonstrator to criticize your technique*, and if necessary, secure a new record which will satisfy him and yourself.

The Influence of the Galvanic Current.¹—The galvanic current has noteworthy effects on the functions of muscle, nerve and cilia,—effects differing at the two poles and also differing as the current is made, running, or broken. These effects and the conditions underlying them can be studied advantageously in a nerve trunk. The facts proved true in nervous tissue are in the main true also for muscle.

Galvani's Experiment.—Between the thumb and forefinger hold in contact a short zinc wire and a short copper wire. Touch the free ends of the two wires to the nerve of a nerve-muscle preparation. Note what occurs. (Galvani, *De Viribus Electricitatis in Motu Musculari Commentarius*, Modena, 1792. Trans. in Ostwald's "Klassiker," 1894.)

This experiment, generally attributed to Galvani, but really performed in all essentials by Swammerdam more than a hundred years before Galvani's re-discovery, is a fundamental experiment, not only in biology but also in physics. If two dissimilar metals such as copper and zinc are connected through a galvanometer, a slight current is observed at the moment the two metals are placed in a weak solution of sodium chloride. The tissues of the body contain aqueous solutions of sodium chloride and other salts. What conclusion may be drawn as to the conditions underlying the result in Galvani's experiment?

***Polarization.**—Attach to each binding post of a dry cell a copper wire to the other end of which is soldered a small clean platinum plate. Let the platinum plates dip into a solution of copper sulphate in a beaker. After five minutes note the change that has occurred on these platinum electrodes.

¹ It is assumed that the student is acquainted with the elementary facts regarding the production of an electric current by chemical action.

The copper sulphate in this experiment is an electrolyte, and the electric current has separated the ions at the two electrodes. The copper kations move toward the kathode, and some of the copper is there deposited. The sulphions (anions) move toward the anode, and on this plate a layer of minute bubbles of oxygen is deposited. When the two plates or electrodes are thus coated and surrounded by separated ions, the system is said to be "polarized."

If these electrodes through which a polarizing current has been passing are quickly connected with a galvanometer, the galvanometer will indicate the presence of a current passing through the copper sulphate solution in a direction opposite to the polarizing current. This is known as the "polarization current."

The same polarization occurs when metal electrodes are used in contact with animal tissues. Polarization introduces errors in physiological observation chiefly by altering from moment to moment the efficiency of the stimulating current. This defect and the possibility of injuring the tissues or producing local currents by metal contacts are avoided by the use of non-polarizable electrodes (see p. 29).

Galvani's experiment proves that the tissue fluids are electrolytes; and observations on polarization of tissues indicate that the passage of the galvanic current through tissues causes a separation of ions of different charge, — anions in the region of the anode, kations in the region of the kathode. The question now arises as to whether this ionic separation is accompanied by differences in physiological states in the neighborhood of the two poles. The answer to this question may suggest not only the nature of electrical stimulation of tissues but also the nature of stimulation by inorganic salts.

**Polar Changes of Irritability.* — Set in a moist chamber, close to the femur clamp, two needle electrodes in cork, and connect them with the secondary coil of an inductorium. Beside the needle electrodes place two non-polarizable electrodes. If there is the slightest possibility of zinc sulphate being on the surface of the porous clay, cover the surface with a fresh layer of kaolin moist with isotonic salt solution. Connect these electrodes to a pair of end posts on a pole-changer with the cross wires in circuit. Attach the middle posts of the pole-changer to a single cell.

Dissect out the full length of the sciatic nerve and the gastrocnemius muscle. Fix the femur in the femur clamp of the moist chamber and arrange to record the contraction of the muscle on a slowly moving drum. Lay the nerve in contact with the four electrodes. The two non-polarizable electrodes should be as far apart as the length of the nerve will permit, but one should be close to the needle electrodes which are next the muscle.

With the drum turning slowly, stimulate the nerve with the weakest interrupted current that will maintain a slight tetanus. In order to do this, it may be necessary to withdraw the secondary coil from the slideway and set it at some distance from the primary. As soon as the height of the partial tetanus is clearly recorded, send for a moment through the nerve an ascending galvanic current, *i. e.*, with the anode near the needle electrodes. Break first the galvanic current and a moment later the tetanizing current. If the results are not perfectly definite, repeat the experiment, using a stronger galvanic current.

Repeat these observations with a descending current, *i. e.*, with the kathode next the needle electrodes. What conclusion may be drawn as to the alterations of irritability caused by the galvanic current in the region of its two poles?

**Polar Changes in Conductivity.* — Arrange the apparatus as in the previous experiment, but place the needle electrodes midway between the two non-polarizable electrodes, and connect the latter through a pole-changer directly with the cell. Introduce into the circuit from the secondary coil a piece of nerve about 2 cm. long, to offer resistance to the passage of the galvanic current through the coil.

Use as a stimulus a tetanizing current barely causing a continuous contraction of the

muscle. While the muscle is in slight tetanus make, for a moment, an ascending galvanic current. Does the muscle now contract?

How is the passage of the nerve impulse affected in the anodal region?

Repeat the experiment with a descending current. How is the passage of the nerve impulse affected in the kathodal region?

Is there an after effect?

How does the alteration of conductivity through the kathodal region differ from the alteration of irritability in the extra-polar kathodal region?

The changes of conductivity to be observed in this experiment do not appear if the galvanic current is not sufficiently strong. In order to produce characteristic polar changes in conductivity it may be necessary to use more than one cell.

Stimulation of Human Nerves.—The agent most commonly used for artificial excitation of human motor nerves is the electric current. In applying an electric current to a human nerve it is impossible to repeat the conditions of the excised nerve in contact with electrodes, and several new factors must be regarded. The current must traverse the skin before reaching the nerve. Only a small part of the current runs from the physical anode along the nerve to the kathode; the rest spreads from the anode in diverging lines through the nerve and through all the neighboring tissues. At the physical kathode these lines again converge. The current, therefore, has a greater density at the points of contact of the physical poles with the skin than anywhere else in the tissues. The stimulating power of a current varies with its density. The skin, moreover, is a poor conductor, and the nerve to be stimulated may be some distance below the surface. Divergence of current lines in tissues, resistance in the skin, and distance of the nerve from the surface may all combine to require a strong current in order that the nerve may be stimulated. But a strong current may stimulate the sensory nerves so severely as to be painful. The problem, therefore, is to pass a strong current painlessly through the skin. This is done first by reducing the resistance of the epidermis by thoroughly soaking the region where the electrodes are to be applied, and secondly by using electrodes sufficiently large to reduce the density of the current in the skin without making the current lines so diffuse as not to stimulate the nerve. In order to make the current less dense in the skin thick metal rods or metal plates covered with cotton wet with salt solution are used as electrodes. When a plate is so large as to cause extensive spreading of the current over the surface of the skin, the current has no noteworthy physiological effect, and the electrode is called an indifferent electrode.

The Determination of "Motor Points."—An earlier experiment has shown that muscles are better stimulated through their nerves than by the direct action of an electric current, because nerves are more irritable than muscles, and because a nerve distributes impulses effectively to all the muscle fibres. There are points on the body surface where the nerves supplying muscles are stimulated more easily than elsewhere. These points are called "motor points." For neurological purposes these points have been charted, but the following method will show the location of some of them without reference to a chart.

Arrange the inductorium for a tetanizing current. Connect to one pole of the secondary coil the brass rod (the stimulating) electrode, to the other the brass plate (the indifferent) electrode. Cover both electrodes with cotton wet with salt solution. Let the left arm rest on the indifferent electrode, start the tetanizing current, and with the stimulating electrode examine the left forearm for motor points. On the anterior surface find a motor point for

the long flexor of the thumb and two motor points for the flexors of the digits. Make a drawing showing the position of these points and other points that may have been observed. Find on the posterior surface three points causing extension and indicate on the drawing their location and the results of stimulating them. Also determine the motor point of the ulnar nerve at the elbow.

Polar Stimulation of Human Nerves.—As previously stated, when an electric current is applied to the body the current lines diverge from the anode and converge towards the kathode. If the physical poles are properly placed at well separated points over a nerve, some of the lines diverging from the physical anode will enter the nerve, pass perhaps for some distance along its length and then emerge, still more dispersed, into surrounding tissues. The lines re-enter the nerve as they approach the kathode, traverse it for some distance, and emerge, again condensed, under the physical pole. The loops of current lines through a nerve in the body give rise to physiological anodes wherever the lines enter the nerve, and to physiological kathodes wherever they leave. Evidently in the anodal region of the nerve, near the physical anode, the current will be less dispersed than in the anodal region near the physical kathode; and similarly in the kathodal region of the nerve near the physical kathode there will be less dispersion than in the kathodal region near the physical anode. Keeping in mind the facts already known, that the stimulus on closing the circuit is at the physiological kathodes, on opening is at the physiological anodes, that kathodal stimulation is stronger than anodal, and that the stimulating effect depends on current density, perform the following experiment.

Join six cells in series and connect the terminal cells through a pole-changer (p. 34), with cross wires in circuit, to a stimulating and to an indifferent electrode. Let the subject hold the indifferent electrode in his hand, while the observer presses the stimulating electrode over the motor point of the ulnar nerve at the elbow. Make the stimulating electrode the kathode, and close and open the circuit. Reverse the current and again close and open the circuit. If there is no response, add another cell to the series and repeat the procedure. Continue thus stimulating the nerve with anodal and kathodal closing and opening stimulations after each addition of a cell. Where did contraction first occur, with anode or kathode over the nerve? on closing or opening the circuit? The following abbreviations are commonly used: KOC, for a contraction on opening the circuit when the kathode is over the nerve; ACC, for a contraction on closing the circuit when the anode is over the nerve; and similarly AOC and KCC. Determine the number of cells required to cause contraction in each of these four cases, and set down in a table the order in which the contractions appeared as the current strength was increased. Explain from previous considerations the reason for this order.

A part of the interest in the stimulation of human nerves arises from characteristic changes in the response to stimulation when the nerve going to a muscle is degenerated. These alterations in the response render the method valuable in diagnosing the pathological state. The alterations are collectively known as the "reaction of degeneration." They are well seen in cases of nerve injury in man. The muscle fails to respond to the interrupted induced current, the response of the muscle to the galvanic current is slow and sluggish, and the order of the response under the physical kathode and under the physical anode, as determined in the above observations, when the galvanic current is gradually increased, is reversed.

SOME GENERAL PHENOMENA OF MUSCULAR AND NERVOUS RESPONSE

Bowditch's classic observations on the heart of the frog led to the first statement of the "all-or-none law," and called attention to the "staircase" phenomenon or "Treppe." These characteristics of cardiac muscle in response to stimulation have proved to be true of other tissues, as can be demonstrated.

The Exposure of the Frog's Heart. — Pith the brain of a frog. If there is bleeding, plug the opening with absorbent cotton. Fasten the animal, back down, on the frog-board. Make a median incision through the skin over the sternum and a transverse incision towards each fore limb. Lay back the flaps. Raise the cartilaginous xiphisternum, and cut along one side of it. On looking beneath, the anterior abdominal vein is seen. Cut across the cartilage anterior to this vessel. Insert under the pectoral girdle the pointed blade of heavy scissors, but hold the point close against the bone to avoid injury to the heart. Divide the girdle. Separate the two parts of the bone by pulling the fore limbs laterally, and fasten the limbs so as to keep the parts thus separated. The heart is revealed beating within the pericardium.

Slit open the pericardium. Note the roughly U-shaped ventricle, and anterior to it the bulbus arteriosus which is filled by each ventricular contraction. Beyond the bulbus are the two aortae. Beneath the bulbus and the beginnings of the aortae are the two auricles. Between the auricles and the ventricle is the auriculo-ventricular groove. Lift up the ventricle. A narrow V-shaped sinus venosus is revealed, marked off from the right auricle by a white crescentic line. Into the posterior end of the sinus the inferior vena cava empties, and into the anterior end, as can be seen by gently lifting the auricles, the two superior venae cavae enter.

What part of the heart contracts first and what is the sequence of the beat in the parts that have been noted — ventricle, bulbus, auricles, and sinus?

What is the change in color when a part contracts? Explain.

The Stannius Ligature. — The automatic contraction of the ventricle can be inhibited by means of a "Stannius ligature." Moisten a ligature thread and pass it under the two aortae. Lift up the ventricle and tie the thread exactly over the line marking the junction of the sinus and auricle. If the ligature is not exactly placed, no change may occur. In that case tie another thread at the same junction. What is the change produced by the tying? (Stannius, *Arch. f. Anat., Physiol. u. wiss. Med.*, 1852, p. 85.)

The All-or-None Law. — After inhibiting the beat of the auricle and ventricle of a frog's heart, fasten horizontally in a clamp the wooden handle of a pithing needle. Set the needle at the sino-auricular junction of the heart. By means of the thread tie tightly to the needle this part of the heart. Attach to the needle a wire and lead it to a post in the secondary coil of an inductorium. Pass a fine copper wire through the tip of the apex, and bend it sharply to form a hook. After setting the frog-board on the wooden stand, connect the hook by means of a silk thread to the heart lever. The cardiac muscle of the frog is more delicate than the gastrocnemius. To lessen the pull on the heart carefully counterpoise the lever with a weight, but be careful not to overweight it; smoke the drum lightly; adjust the writing point so that it presses very gently upon the smoked surface. Use great care to *prevent injury from drying*. Attach to the other post of the inductorium the wire passing through the apex.

Find a break shock that will cause the heart to contract. Record the contraction. Turn the drum 2 mm., slide out the secondary coil 1 mm., and after waiting 15 seconds stimulate again. Continue thus until the heart fails to respond.

Does the height of the contraction under these conditions vary with the strength of the stimulus? Compare this result with observations on skeletal muscle. (Bowditch, *Ber. d. k. sächs. Gesell. d. Wiss.*, Leipzig, 1871, p. 682.)

The Recovery of Excitability Following a Response. — In the preceding experiment you were cautioned to wait 15 seconds between successive excitations. The following experiment will show why.

Use the same preparation. Insert a signal magnet in the primary circuit and arrange the inductorium so that the make shock is just adequate to excite. With the drum rotating very slowly stimulate with pairs of induction shocks, the make following the break at gradually decreasing intervals (3 seconds and less) until no response is obtained. The interval between the pairs of stimulations should be constant and at least 15 seconds in length. The period following one excitation during which a second excitation is ineffective is called the "refractory period." It is an important phenomenon not only in cardiac muscle but also in skeletal muscle and nerve. The changes in irritability following one excitation can best be studied in cardiac muscle because recovery takes place slowly. Using the foregoing preparation and technique they can be measured with some accuracy.

Measurement of Changes in Irritability. — Use a fresh preparation if necessary, arranged as before. Start with the inductorium in a position such that the make shock is just effective when applied to the fully recovered muscle. Stimulate with a break shock followed by a make shock after an interval of 7 seconds. Keeping the interval at 7 seconds increase the distance between the primary and secondary coil one millimeter at a time until the make shock becomes ineffective. Be sure the binding screws on the slider of the inductorium are tight each time. A rest interval of at least 15 seconds should separate the pairs of excitations. Record the position at which the make shock is last effective with a 7 second interval.

Repeat, reducing the interval to 6, 5, 4, 3, 2, and 1 seconds, and fractionating the intervals further if you can do so. Plot the positions of the secondary coil, which may be taken as a measure of the excitability of the cardiac muscle, against the corresponding time intervals. Can you distinguish

The Absolute Refractory Period?

The Relative Refractory Period?

The Period of Hyperexcitability?

Explain the normal rhythmicity of the heart.

The Staircase Effect (or Treppe). The response as well as the excitability of cardiac muscle is influenced by immediately preceding events. Devise an experiment which will demonstrate that for a short interval following one response a second response is abnormally great. Measure the interval during which this is true. Can you relate it to any definite part of the curve of recovering excitability? Can you reconcile it with the all-or-none law?

The Effect of a "Tetanizing" Current. — Record on a slowly turning drum, the effect of stimulating the ventricle for a short interval (5 seconds) with the weakest effective tetanizing current.

Is the response continuous or intermittent?

Is the stimulus continuous or intermittent?

What is the effect on the rate as the current is made stronger? Explain.

The All-or-none Effect in Skeletal Muscle. — Arrange the inductorium for single shocks, with a mercury key in the primary circuit. The key may be operated by dipping the amalgamated end of one primary wire in a mercury bath which is in contact with the other wire. With weak currents

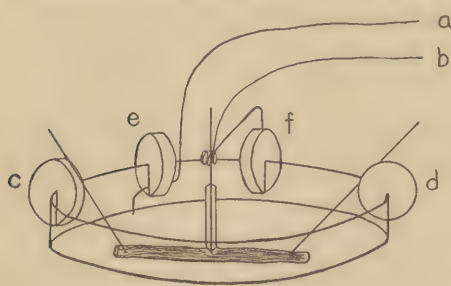


FIG. 3.

this gives requisitely uniform breaks. Lead the secondary wires to a pole-changer (see p. 34), and extend from this the two very fine stimulating wires, *a* and *b*, Figure 3.

Remove a sartorius muscle, detaching it at the tibial insertion and reflecting it upward as its delicate connections are cut, finally severing it at the pelvic origin, after detaching the overlying portion of the rectus abdominis. Lay the muscle, ventral surface up, in a small Petri dish and anchor it securely against the bottom with needle-hooks attached to opposite sides of the rim by cork hold-

ers, *c* and *d*, being careful not to stretch the muscle beyond its original length. Shake a bottle containing powdered charcoal and, removing the cork, blow a little of the escaping fine carbon dust upon the muscle. Finally, cover the muscle completely with Ringer's solution.

Dip one stimulating wire freely in the solution as an indifferent electrode and fix it to the side of the dish with a cork holder, *e*. The other wire is clipped into the coiled electrode arm which holds the active electrode. This electrode is a very fine needle, insulated with sealing wax except at the extreme apex of its point, the apex thus forming a conductor of extremely small sectional area. The electrode arm is inserted in its cork support, *f*, and affixed to the rim of the dish in such a position that the electrode point just touches the muscle near the pelvic end. Place the preparation on the stage of the microscope so that any portion of the distal region of the muscle may be brought under the low-power objective by moving the dish.

Observe the muscle through the microscope and beginning with the weakest possible induced current increase the strength of the stimulus until the muscle just begins to contract. If the carbon particles under observation move *transversely* or obliquely to the direction of the muscle fibres it means that the field chosen for observation does not include the particular fibres which are being stimulated, but only such as are being passively pulled aside by the contraction of their neighbors. Actively contracting fibres which move the carbon particles lengthwise of the muscle are wanted for observation, and the dish, or perhaps the stimulating electrode, must be moved until such fibres are brought under observation. If necessary, cautiously decreasing the strength of stimulus, find the position of the pole-changer at which the muscle responds only to the weakest effective break shock. This determines that the stimulating electrode is a cathode. Why? This is the direction of current flow which is wanted throughout the rest of the experiment. Why?

Make sure that the stimulus is of just threshold intensity for the fibre or fibres under observation, *i. e.*, any weaker stimulus by so much as half a millimetre of coil distance causes no contraction at all. Move the secondary coil toward the primary a half millimetre at a time, and stimulate for each position of the coil with a make and break shock. Giving an arbitrary value to the scale intervals, record the extent of responses to break shocks. When contraction becomes too general to follow accurately, reverse the process. Plot a curve of contraction change, with half millimetres of coil movement as abscissa.

Compare the relations of change of stimulus to variations in size of contraction when few fibres are excited, and when many fibres are in action.

Explain fully why the results lead to an all-or-none interpretation of the contraction in skeletal muscle. (Pratt and Eisenberger, *Am. J. Physiol.*, 1919, xlix, 1.)

Properties of the Nerve Impulse Shown by Narcosis. — Select a large frog. Make two nerve-muscle preparations, dissecting the two sciatic nerves as nearly at the same time as possible with great care to avoid injuring them in any way. The gastrocnemius muscle need not be dissected out; use the "demonstration preparation," with the foot cut off at the ankle to render it less bulky. Immerse both preparations in cold-blooded Ringer's solution and leave till the apparatus is ready. Arrange the inductorium for single shocks; connect the terminals of the secondary coil with the binding posts on the Adrian (J. Physiol., 1912, xiv, 393) narcotizing chamber (see Fig. 4). Note that there are four notches in a straight line opposite one pair of platinum electrodes, and two notches opposite the other. Place this chamber on a

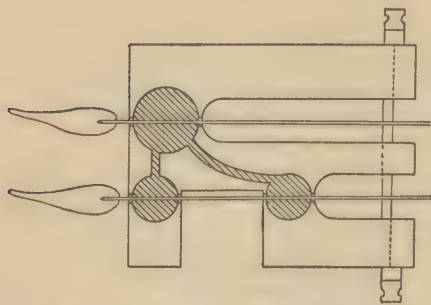


FIG. 4. — Adrian narcotizing chamber.

clean dry glass plate, and with wax prevent the chamber from shifting its position. Have ready the glass bell jar used with the ordinary moist chamber, with wet filter paper inside.

Remove the two nerve-muscle preparations from the Ringer's solution; lay the muscles on the plate, lead the nerves carefully through the two rows of notches and lay their central ends across the platinum electrodes. Each nerve should lie in the bottom of every notch through which it passes and must make contact with both electrodes of the pair on which it is laid. Great care must be used to avoid stretching or jamming the nerves against any part of the apparatus. They must not be allowed to dry, nor must saline solution be applied after they are in position. Place the bell jar over the preparation and stimulate with a single break shock. A position of the secondary coil should be found at which both muscles give maximal contractions from the break shock and none from the make. Keep the secondary coil in this position throughout the experiment. Remove the bell jar and carefully, with a pipette, introduce into the connected group of cups in the narcotizing chamber Ringer's solution containing 10% alcohol. It should be introduced until the nerves are completely submerged between the notches, but it must not be permitted to leak out; the general level can be raised above the lowest points in the notches without leakage, on account of surface tension. This should be done without unnecessary delay but with great care, for a single drop touching the nerve outside of the cups will spoil the experiment. Replace the bell jar over the preparation.

Stimulate with a break shock every 15 or 20 seconds, watching the contractions of the muscles, until both muscles have ceased to contract.

Which muscle ceases first?

Just after it has ceased contraction, try the effect of very rapidly repeated stimulation. Does the muscle again respond?

Measure the distances that the nerve impulses had to traverse in the narcotized regions. Is the length of nerve subjected to narcosis a determining factor in the abolition of the impulse?

Does this indicate that the impulse is extinguished immediately on entering a narcotized region of nerve or by progressive decrement?

Note that the nerve passing through the two short cups was subjected to narcosis over a greater total length than the other. Does the intervening normal area influence the result? How?

How do you reconcile decrement with the all-or-none nature of conduction?

Is the energy of the propagated disturbance supplied by the stimulus or the nerve fibre?

CONTRACTION OF MUSCLE AND CILIA

I. SKELETAL MUSCLE

THUS far the organs studied have been muscles with their nerves attached. If a muscle is to be studied by itself, it must be separated from its nervous control; otherwise an impulse started at one point will be carried at once throughout the body of the muscle by the nerve fibres branching in its substance. When the nerve going to a muscle is cut, the nerve fibres soon degenerate and the muscle is thus left independent of its nerve supply. This procedure, however, is attended with delay. A shorter method of isolating a muscle from nervous influences is desirable.

***The Blocking of Nervous Impulses by Curare.**—Pith the brain of a frog.¹ Make a short slit in the skin on the dorsal side of the left thigh and, with care not to injure blood vessels, lay bare the sciatic nerve. Under the nerve pass a ligature wet with isotonic salt solution and tie it tightly around the thigh in order to shut off the blood flow. Cover the exposed nerve with filter paper wet with isotonic salt solution. Inject 3 or 4 drops of curare solution under the skin of the back. In 10 or 15 minutes the frog should become limp. When the frog, suspended by the lower jaw, no longer retracts the right limb on mechanical stimulation of the right foot, expose both sciatic nerves up to the vertebral column. Stimulate the right sciatic near the column with a submaximal stimulus (why submaximal?). Do the muscles of the right leg contract?

Stimulate the left sciatic similarly. Do the muscles of the left leg contract?

Has the nerve trunk been affected by the curare?

Remove the skin over the right gastrocnemius and apply the electrodes to the muscle. Does it respond to stimulation? Is muscle independently irritable?

Where has the curare affected the neuro-muscular mechanism? (Bernard, *La Science Expérimentale*, Paris, 1878, p. 294.)

The Single Contraction

The Duration of the Three Periods.—Arrange a gastrocnemius muscle (without its nerve) to write a record of its contraction on a drum. Load the muscle with the weight pan, which weighs approximately 5 grams, and a 5-gram weight. Place in the primary circuit, in addition to a simple key, an electromagnetic signal. The electromagnetic signal should be supported on the same stand as the muscle lever, and its writing point should be *close to the writing point of the muscle lever* and as nearly as possible directly below it. On a separate stand fasten a tuning fork vibrating vertically. A writing point, attached to one bar of the tuning fork, should point backwards toward the left and should be adjusted to write its record between the signal and the muscle curve. By means of the screw at the top raise from the friction support the sleeve bearing the drum and turn down the set screw. Bring the writing points of the muscle lever and the signal against the drum. Set the tuning fork vibrating and bring its writing point against

¹ Since curare in the amounts here used may not abolish sensation, always take the precaution of destroying the brain before curarizing.

the drum between the other two writing points. While one starts the drum spinning by hand, another stimulates the muscle with a maximal make induction shock. As soon as the muscle has relaxed, withdraw the tuning fork and stop the drum before it has begun a second revolution. The speed of the drum should be such as to make the distance between the time waves about equal to their height.

In order to determine the temporal relation of points on two records written at the same time it is always necessary to have simultaneous ordinates. Without changing the apparatus (why?) turn the drum until the writing point of the signal is precisely at the position where it began to leave the base line. This position marks the time when the current was made. With the drum stationary make the current again. The upward movement of the muscle lever marks the point in the muscle record at which the current entered the muscle. Establish another ordinate on the base line at a point corresponding to the highest point in the muscle curve. By means of these ordinates it is now possible to determine the duration of the three periods of the curve.

The first period lasts from the point of stimulation to the point of commencing contraction and is known as the "latent period." After the record has been made permanent, measure the duration of this period by drawing from these points through the tuning-fork record lines perpendicular to the abscissa of the muscle curve. From crest to crest the tuning-fork record measures one hundredth of a second. State three factors concerned in the production of the "latent period."

The second period in the single contraction lies between the point where shortening commences and the point where shortening is greatest. It is called the "period of contraction." In what portion of this period is the curve convex to the abscissa? In what portion is it concave to the abscissa? What do these changes of curvature indicate as to the rapidity of development of the contracted state at different times during this period of contraction?

The third period lies between the highest point of the curve and the point at which the curve again touches the abscissa and is known as the "period of relaxation." Examine the varying curvatures of this portion of the record and state their significance as to the varying rate of relaxation of the muscle during the period.

Before disturbing the apparatus obtain the necessary data for calculating the amount of shortening of the muscle in the experiment. What percentage of the length of the muscle is the shortening?

Measure the duration of the period of contraction and the period of relaxation.

What errors in the record may arise from the weight of the muscle lever?

Should the lever be light or heavy?

What factors determine the proper length of a lever?

The Isometric Contraction and the Influence of Increasing Initial Tension. — In the observations on contraction thus far the muscle has worked under fairly uniform tension. The only alteration in tension has been due to the inertia of the weight at the beginning of the period of contraction and the acceleration of the weight towards the end of the period. Contraction with uniform tension is isotonic. In the body the altering advantages and disadvantages in lever movements of the bones during muscular shortening, and the opposition of their opponents, place the muscles under widely varying tensions from moment to moment. It is desirable, therefore, to know the effects of varying tensions on contraction. To ascertain these effects the muscle is caused to contract against a spring; since the spring does not

permit any considerable shortening of the muscle, the muscular energy is converted into tension. Contraction under these circumstances is said to be isometric.

Fasten a flat-jawed clamp in the rigid muscle lever. Let the clamp support the femur of a gastrocnemius preparation so that the tendon of the muscle hangs over the hook attached to the spring. Connect the muscle firmly to the spring by means of an S-shaped pin passed through the tendon. Raise the clamp until the muscle rests in place without tension. When the muscle contracts against the spring the muscle should shorten less than a millimetre. With the drum stationary stimulate the muscle with a maximum make induction shock. Secure several records of this isometric contraction.

Write a short abscissa with the muscle at its resting length. *Without lifting or dropping the drum*, raise the flat-jawed clamp slightly, in order to place the muscle in a state of tension, and write a second short abscissa. The distance from the first to the second abscissa measures the tension under which the muscle will begin its contraction. Secure a record of an isometric contraction with exactly the same stimulus as before. Repeat this procedure with the muscle each time under slightly greater initial tension, until no contraction occurs.

Detach the muscle, and overturn the spring. Let the writing point draw a zero line on the drum. Attach to the hook of the spring the weight pan and add weights to make a pull of 50 grams. Turn the drum and draw a line below the zero line, recording the extension of the spring by this weight. Repeat this procedure with 100, 150, 200 grams, and more if necessary. Thus the tension of the spring is standardized; and by applying this standard to the isometric records it is possible to know in grams the tension which the muscle reached during the isometric contraction.

After the records of the contractions have been made permanent, calculate the maximum shortening of the muscle in the isometric contraction. Set down in a table the figures which show the initial tension in grams and the maximum tension in grams. What has been the effect of changing the initial tension on the maximum tension which is developed by the muscle?

From the figures which this experiment has provided it is evident that the pull exerted by a muscle is not determined solely by the strength of the stimulus, but is affected by the tension under which the muscle contracts before and after it has begun to respond to the stimulus. The ability of skeletal muscles to adjust the liberation of their energy to the work to be performed is one of many examples of the adaptation of biological mechanisms to variations in the demands placed upon them. This automatic increase of contractile energy when opposed is probably of great value in adjusting muscular contractions to their constantly varying stress.

The Influence of Increasing Load. — As already noted, a muscle may contract and raise a load it is already supporting, in which case it is said to be loaded; or it may begin to lift only after it has begun to contract, in which case it is said to be after-loaded. In the body muscles are constantly loaded by the pull of their antagonists, but usually the main load — the weight to be lifted — is applied only after they have begun to contract, *i. e.*, the muscles are both loaded and after-loaded. The experiment on the isometric contraction has shown that a muscle will do a greater amount of work if it contracts with an initial tension. This is evidently the case in the body. It is desirable, however, to see what the effect may be of increasing the load without increasing the initial tension.

Make a gastrocnemius preparation and fasten the femur in a flat-jawed clamp so that the tendon hangs over the hook in the lever of the rigid tripod. For convenience the straw on

the muscle lever should be cut so that the distance from the axis to the writing point is a simple multiple of the distance from the axis to the hook. Adjust the after-loading screw so that the muscle is at its resting length. With the drum stationary record the contraction of the muscle stimulated by a maximum make induction shock. Attach to the lower hook on the muscle lever the weight pan and add weights to 20 gms. Turn the drum about a half centimetre and stimulate as before. Repeat the process with 40, 60, 80 gms., and so forth until the muscle is unable to raise the load from the after-loading screw. At this point raise the flat-jawed clamp until the muscle is slightly stretched by the load. Does a repetition of the former stimulus now cause the muscle to contract?

Before dismounting the apparatus, make the measurements necessary for the calculations mentioned in the next paragraph.

When the records of the contraction have been made permanent, calculate the actual distances to which the different weights have been raised by the muscular contraction. What effect has increasing load on the height of the contraction?

***The Influence of Increasing Load on Work Done.** — The previous experiment has given data as to the weight lifted, in grams, and the distance through which the weight was lifted, in millimetres, with an increasing load. From these data calculate the work done in gram-millimetres for the different loads which were used. On coördinate paper plot a curve of the height of contraction with increasing loads. On the same abscissa plot another curve of the work done with increasing loads. How does increasing the load affect the work done by the muscle?

At what region in the gradual increase of the load is the greatest work performed?

What conclusion do you draw as to the load-factor in the optimum condition for the performance of mechanical work?

***The Influence of Temperature on Contraction.** — The processes underlying biological activity are largely chemical. The rate of chemical change is markedly affected by temperature variations. It is important to know, therefore, how biological processes are affected by heat and cold. These temperature conditions are especially important to animals which have the temperature of their surroundings (the so-called "cold-blooded" animals); they are also important to warm-blooded animals, for high body temperature may disturb the tissue proteins.

To the upright rod of an iron stand clamp a muscle lever. Above the lever set a pulley rod with one side of the pulley over the double hook on the lever; below the lever attach a flat-jawed clamp holding an L-shaped glass rod, the point of which should be under the double hook on the lever. On the same stand clamp an iron ring-support, and place on it a piece of wire gauze. The iron ring and gauze should be at such a height that a Bunsen flame can be applied beneath them.

Make a gastrocnemius preparation. By means of a fine copper wire fasten the femur firmly to the end of the L-shaped glass rod, and continue the wire to the binding post on the flat-jawed clamp. With a needle pass a silk thread through the tendon and tie. Pass the thread over the pulley and directly down to the double hook on the muscle lever. Fasten around the tendon also a fine copper wire; attach the wire, which should be slack, to the post on the lever. Load the muscle with the weight pan and 10 grams. Arrange to stimulate the muscle with a maximum induction shock.

Immerse the gastrocnemius in a beaker of ice-cold Ringer's solution placed on the wire gauze. With a thermometer determine the temperature of the solution. The observation should start at or near 0° C. When the muscle has been exposed to the cold for three minutes, lower the ring support until the upper part of the muscle projects above the level of the solution. With the tuning fork vibrating, start the drum at its most rapid rate, and let the muscle immediately describe its curve in response to a maximum make shock. Quickly secure another record, and then surround the muscle again with Ringer's solution.

Remove the ice from the larger beaker and add water. Warm the water until the temperature of the salt solution is brought to 10° C. Using the same method as before, secure two records of the contraction at this temperature. Repeat the observations for 20°, 30°, and then record on a slowly moving drum what occurs as the salt solution is *very gradually* heated to 60°. Stimulate the muscle again. What happens?

Describe the appearance of the muscle.

How does it feel?

Remove any adherent salt solution by means of filter paper, cut the muscle transversely, and test the chemical reaction of the cut surface by means of litmus paper. Compare this reaction with that of fresh muscle.

After the smoked paper has been shellacked cut out pieces of uniform size, each bearing a recorded contraction. Arrange these records in the note-book in the order of increasing temperature. Set down in a table the effect of temperature on contraction time, relaxation time, and height of contraction. Summarize in a statement the general effect of changes of temperature on muscular contractions. State any variations from the general rule.

The change which took place in the muscle when heated above 40° was heat rigor. It is accompanied by the coagulation of the muscle proteins. The proteins of warm-blooded animals do not coagulate at so low a temperature as those of cold-blooded animals, but some of the proteins of mammalian nerve cells undergo visible changes at a continued temperature of 42°. The importance of preventing a body temperature of this degree is obvious.

***The Influence of Chemical Agents.** — In observations already made, the effects of distilled water and of strong salt solutions in stimulating muscular contraction have been seen. A chemical agent affecting contraction in a peculiar way is the drug veratrine.

Prepare the hyoglossus muscle as follows: In a pithed frog cut through the joint between the two jaws on either side and extend the incision to the shoulder girdle. Pull the lower jaw slightly forward and free it by a transverse cut at the forward edge of the shoulder girdle. Lay the jaw on a glass plate, tongue side upwards. The hyoglossus runs from the hyoid cartilage to the apex of the jaw and thence backward to the tip of the tongue. Turn the tongue forwards beyond the apex of the jaw and tie a fine copper wire around the tip. Clamp the hyoid bone with skin and mucous coverings in the flat-jawed clamp. With a tuning fork recording, let the muscle write its contraction on a moving drum in response to a single induction shock. Introduce in the lymph space beneath the skin five drops of a 0.1 per cent solution of veratrine acetate and after a few minutes secure records of the contraction with the same strength of stimulus. If the period of relaxation has been greatly lengthened, repeat the stimulation several times to see if the veratrine effect disappears. If the effect disappears, let the muscle rest a few minutes to see if the effect returns.

The Continuous Contraction

In the study of physiological processes the complexity of the activities requires, so far as possible, a simplification of the conditions of observation. It is of the first importance, therefore, to produce in the organ to be studied an uncomplicated characteristic response which can be uniformly repeated. It is then possible to study the factors which modify the response. Thus far in the study of muscle this method has been followed; the muscle has been caused to exhibit a simple contraction, the single twitch, and the effects of various agents on the single twitch have been observed. This simple, well-defined activity is not, however, the characteristic activity of muscles in the body. The body muscles do not normally contract in single twitches, but in a more or less prolonged continuous shortening. The next inquiry is concerned with the manner in which the normal sustained contraction is brought about.

The Effect of Decreasing the Interval between Two Stimuli. — Use the same arrangement as in the previous experiment, but after-load the muscle with 40 gms. and set close to the writing point of the muscle lever an electro-magnetic signal connected in the primary circuit with a simple key. Loosen one of the wires screwed to the simple key and attach it to a needle.

With the drum revolving at rapid speed touch the needle to the bar of the key and thus stimulate the muscle with a maximal make shock; as soon as the muscle has relaxed, lift the needle and thus stimulate with a maximal break shock. Secure a number of these pairs of contractions, but gradually decrease the interval between the two stimuli. The later make and break shocks can be brought gradually closer together by drawing the point of the needle across the bar of the key each time with greater speed. By means of simultaneous ordinates determine at what point in the first contraction the second stimulus was sent into the muscle.

At what point did the second stimulus cause the highest summed contraction?

Is the height of the second contraction, measured from the level from which it started, greater in any case than the height of the first contraction?

The important result to be noticed in this experiment is that, when a muscle is stimulated a second time before the periods of contraction and relaxation are ended, the muscle does not finish the process started by the first stimulus but at once takes on a new activity in response to the second. And if the second stimulus is properly placed, the muscle merely continues the period of contraction which the first stimulus started. This result suggests the possibility of a continuous contraction being caused by stimulations so rapidly repeated that the muscle does not have time to relax between successive stimuli.

The Development of a Continuous Contraction (Tetanus). — Using the same arrangement of apparatus and muscle as in the previous experiment, repeatedly make and break the primary circuit by tapping with the simple key. In the first experiments tap the key so slowly that successive stimuli come to the muscle when relaxation is nearly complete. In a series of such stimulations gradually increase the speed of tapping until the maximum is reached.

As the interval between successive stimuli decreases what is the effect on successive contractions of the muscle?

Arrange the primary circuit to produce a tetanizing current in the inductorium, and stimulate the muscle. The very rapid stimulation causes the successive contractions to fuse together so completely that the curve of contraction becomes a continuous line.

The Nature of Voluntary Contraction. — Note that our ordinary muscular movements are not twitches, but continuous contractions. It follows, therefore, that our skeletal muscles are probably stimulated by rapidly repeated impulses coming through their nerves. That the muscle fibres under these circumstances contract rhythmically is indicated by the musical note emitted during contraction.

Press the palm of the hand against the ear, and after hearing the resulting sound, strongly contract the biceps and observe the new deeper note that results. A stethoscope placed over a contracting muscle will reveal the same sound.

***Artificial Tetanus of Human Muscle.** — Clamp the L-shaped ergograph to the desk. Let the adjustable rod rest on the index finger and reach to the horizontal spring. Secure a record of the contraction of the abductor indicis stimulated by a tetanizing current. In order to do this place on the forearm the flat brass electrode covered with cotton wet with salt solution. Touch the brass-rod electrode, with the point similarly covered with moist cotton, to the skin over the abductor at a point near the angle between the first and second metacarpals. Stimulate the muscle and compare the resulting curve with the tetanus of frog muscle and with voluntary tetanus.

Increase and Decrease of Muscular Efficiency from Repeated Stimulation

Common effects of repeated stimulation of muscle are, first, an increased efficiency, and later, a decreased efficiency of the muscle as a mechanism for doing work. The first, or "staircase" effect underlies the custom of "warming up" before an exertion; the second is known as "fatigue." The next two experiments should be combined.

The Staircase Effect and Contracture. — Arrange a gastrocnemius muscle after-loaded with 10 gms. to trace its contraction on the smoked drum. Adjust the secondary coil so that both make and break shocks cause a maximal contraction. The muscle should be very fresh and as little stimulated as possible before the experiment is begun. With the drum moving at its slowest speed stimulate the muscle rhythmically at intervals of a second for five minutes.

Do the records of the first contraction gradually increase in height? If so, the effect is known as the "staircase" effect. It is an example of increase in irritability of an organ resulting from the activity of the organ. Evidently this increase of irritability leads to an increase of efficiency without any change in the strength of stimulation. Cite from your own experience other examples of this effect.

Harmonize the "staircase" effect with the "all-or-none" law.

As the stimulations were repeated in this experiment was there a gradual rise of the line to which the muscle relaxed?

If so, and the stimulations were repeated rhythmically, what must be the explanation?

Fatigue of Excised Muscle. — Continue to stimulate with maximal shocks, while letting the drum revolve very slowly.

A fatigue curve such as is recorded under these circumstances has four features: (a) introductory contractions, (b) the "staircase" effect, (c) contracture, and (d) gradually diminishing height of the separate contractions — the characteristic feature of fatigue.

After fatigue has become complete allow a rest of five minutes and then stimulate as in the first curve.

Is restoration of function thorough ?

What conditions of experimentation would result in greater recovery ?

Repeat these observations with the remaining gastrocnemius muscle, interrupting the curve at the period of greatest muscular efficiency. Allow a rest of ten minutes and then endeavor to duplicate the curve.

Is the muscle restored to its original condition ?

Excised muscle must contract solely at the expense of its own store of energy. In the use of this store, chemical products accumulate which check contraction. Suggest an explanation of recovery from fatigue.

***Comparison between the Development of Fatigue in Muscles with and without Blood Supply.** — Anaesthetize a frog with urethane (1 c. c. of a 10 per cent solution for 50 grams of frog, injected into the dorsal lymph sac). When the animal is inert, fasten him, stretched full length, with back down, to the frog board. Support the body against the pull of the muscle by means of a peg set against the pubis between the legs. Free the gastrocnemius tendons through small incisions in the skin of the ankle, being careful to avoid hemorrhage. Attach to one tendon a muscle lever carrying as an after-load a weight of 30 gms. This is accomplished by passing the strong connecting thread around two pulleys held firmly in iron clamps. Reduce friction as far as possible by careful alignment of the apparatus. The lever should be after-loaded as in the previous experiment.

With kymograph in place and with preparations made to stimulate the muscle directly with maximal shocks at 30 per minute, pass a linen ligature about the thigh, and tie off the circulation in the leg. Start the record at once.

On finishing a complete fatigue curve shift the apparatus to the tendon of the other leg, in which the circulation has not been interrupted, and make a second curve. When this tracing shows a uniform fatigue level below which contractions are falling very slowly, stop stimulation.

Explain the fatigue level finally attained. Increase the rate of stimulation. Does it alter this condition ?

Make a record of the effect of rest, comparing the muscles with and without circulation.

In the case of the muscle with normal blood supply does contracture occur ?

Is the staircase effect marked ? If not, why not ?

Compare the effects of rest with and without good circulation.

***The Effects of the Contractile Process**

The contraction of muscle, when loaded, results, as already seen, in a performance of mechanical work. Aside from the change of form, the doing of external work is the most obvious effect of muscular activity. But contraction is attended by other important effects. The ability to do work implies a store of energy. To release that energy chemical change must take place. During contraction, therefore, the muscle is the seat of chemical alterations, and these are oxidative in character. These alterations are considered in connection with the metabolism of work (p. 142), and in lectures. Attending muscular contraction there is also a development of heat; this effect of contraction is considered in connection with the regulation of temperature. Still another phenomenon accompanying contraction is the production of an

electromotive force in the active muscle. Of the four effects of stimulating muscle — mechanical work, chemical change, heat production, and electrical phenomena — only the first and the last will be considered at this time.

Mechanical Work. — In the experiment on the effect of load, data were secured which permitted the calculation of the work done by a muscle lifting increasing loads. The work done in that case, however, was not effective, for the weight was restored in each case to the position from which it was lifted. The pull of the weight during muscular relaxation can be shown to restore to the muscle, as heat, the amount of energy exhibited by the muscle in originally raising the weight. Thus, although the muscle does internal work, it fails to effect any external work. In order to study the actual capability of excised muscle to perform external work, with as little disturbance as possible from tension during the period of relaxation, the work adder was devised. (Fick, *Untersuchungen aus dem Züricher Laboratorium*, Vienna, 1869.)

(* *Demonstration.* — *The Work Adder.* — A double semimembranosus-gracilis preparation is made, with bony attachments. The two pairs of muscles are held together by the pelvis. One end of the preparation is fastened in the flat-jawed clamp; the other end attached to the counterpoised lever of the work adder. When the muscle contracts the lever rises and by means of a pawl turns a wheel; when the muscle relaxes the lever falls, for it has a slight weight, but the wheel is prevented by another pawl from turning back with the lever. A weight attached to the wheel would, therefore, be raised during contraction and would remain supported in its new position during relaxation. The muscle thus during the period of contraction alone does effective external work.

The long thread hanging down from the wheel is loaded with 20 gms. The distance of the weight from the axis of the wheel is measured. At intervals of one second the muscle is stimulated with maximal induction shocks until it ceases to contract. Calculate the total work done by the muscle.)

Electromotive Phenomena. — Almost every manifestation of bodily activity is accompanied by the development of an electrical difference of potential. Indeed, when a nerve impulse passes, the only sign of a physical or chemical accompaniment of the physiological process is the electrical change. And in certain fish are found electric organs whose special function is the production of an electromotive force. It is of interest to study further the conditions under which differences of electrical potential appear in organisms. Since such differences tend to be equalized by the passage of a current, great care should be taken in work on electromotive phenomena to avoid in the neighborhood of the structure to be studied any tissue fluids, salt solution, or filter paper wet with salt solution. Use merely a *clean, dry plate* to support the tissues.

Capillary Electrometer. — To demonstrate differences of electrical potential a galvanometer or electrometer is used. The most convenient instrument in many respects is the capillary electrometer. In essentials this consists of a very fine tapering capillary glass tube, holding clean mercury and dipping into 10 per cent sulphuric acid contained in a glass cup. Below the mercury the capillary tube also is filled with acid. To make the instrument more sensitive the capillary tube is connected with an arrangement which permits the mercury to be put under pressure. Platinum wires lead out from the mercury column and from the mercury in the bottom of the acid cup, and these wires are connected by a short-circuiting key. The key should be kept closed whenever the instrument is not being used for observation.

If the point of higher potential is known, the mercury column should be connected with that point, and the acid cup with the point of lower potential. On breaking the contact at the short-circuiting key the difference of potential causes a diminution of the surface tension of the mercury; the column drops to a narrower place in the tube where the weakened surface tension is again able to hold in equilibrium the forces tending to move the mercury downward. A current in the opposite direction has an opposite effect; in other words, the meniscus moves in the direction of the current. These movements are best observed with the low-power objective through the microscope. The electrometer must never be laid flat, and should be placed on the stage of the microscope only after the stage has been tilted to a nearly vertical position. If, after pressure has been applied to the mercury, the meniscus is below the field of the objective, the capillary tube may be gently raised. Continued use is likely to result in diminution of sensitiveness; a fresh surface can be secured by driving some of the mercury through the capillary tube and then reducing the pressure to restore the meniscus to its former position.

Metals in contact with tissues are capable of giving rise to an electromotive force sufficient to stimulate (see Galvani's experiment, p. 12). For this reason, in observations on electromotive phenomena in living structures, metal electrodes are avoided and only non-polarizable electrodes are employed.

Non-polarizable Electrodes. — Pure zinc electrodes or imperfectly purified zinc electrodes, if amalgamated¹ are not polarized when used in a strong zinc sulphate solution. This fact is utilized in making non-polarizable electrodes. Such electrodes have been devised in various forms. They consist essentially, however, of the following parts: a receptacle having a porous base wet with isotonic salt solution, in the receptacle a strong solution of zinc sulphate which is in electrical contact with the salt solution, and rods of pure zinc or amalgamated zinc dipping in the zinc sulphate solution. The porous substance wet with isotonic salt solution is the electrode which is placed in contact with the tissues. During the relatively short period of use of such an electrode the porous substance acts as a barrier between the tissue and the zinc sulphate. Thus there is no polarization at the metallic surfaces through which the current passes and there is no foreign element brought in contact with the stimulated tissues.

In using non-polarizable electrodes great care should be taken to avoid spilling the zinc sulphate on parts likely to touch the irritable tissue, for the zinc sulphate is highly injurious. If boot electrodes, the porous portion of which consists of hardened clay, are used, the receptacle for zinc sulphate should be carefully emptied and then thoroughly washed out with running water after each period of use. They may then be placed in two or three hundred cubic centimetres of isotonic salt solution and left to soak until again needed. If there is any possibility that the zinc sulphate has come in contact with the surface on which the irritable tissue is to rest, that surface should be coated before use with kaolin moistened with isotonic salt solution. With such electrodes the action of the galvanic current can be studied without alteration of the strength of the current and without injury to the tissues.

Keep the thoroughly washed electrodes in isotonic salt solution, ready for use. When in use each electrode is supported on a metal rod by means of a spring clip.

Whenever these electrodes are used in the study of electromotive phenomena, it is essential to make sure that they are iso-electric. After being prepared, they are connected with the capillary electrometer; with the clay tips in contact with each other there should be no movement of the meniscus when the short-circuiting key is depressed. If there is a movement, the electrodes are not iso-electric. In that case they must be freshly washed or covered with kaolin moist with isotonic salt solution.

(Demonstration. — The Current of Action and the Current of Injury. — Carefully remove a gastrocnemius muscle with its nerve. Lay the muscle over the tips of two iso-electric non-polarizable electrodes; the belly of the muscle should be on one, the cut tendon on the other. Connect the muscle with the capillary of the electrometer. If the meniscus does not move when the short-circuiting key is depressed, touch the muscle near the tendon with a hot wire. In which direction now does the meniscus move? Where is the point of higher potential in the muscle, at the belly or at the injured tendon? The difference of potential in injured tissues gives rise to a "current of injury."

Stimulate the muscle through its nerve with single induction shocks and note the movement of the meniscus with each stimulation. In which direction does the meniscus move?

Where is the point of higher potential in the stimulated muscle, in the active part, or at the inactive tendon?)

The Rheoscopic Nerve-muscle Preparation. — Make two nerve-muscle preparations — one of them for demonstration — and lay them on a clean dry plate. Loop the nerve of the demonstration preparation so that it crosses the isolated muscle twice. As far as possible from this muscle stimulate its nerve with a submaximal single induction shock. What change is seen in this “first” muscle, and in the demonstration preparation? Now stimulate the nerve with a weak tetanizing current. Again note the activity of the two muscles. Tie a ligature tightly about the stimulated nerve near the muscle and once more stimulate at the same point and with the same current as before. If nothing now results, clearly the phenomena previously observed could not have been due to spreading of the electric current, yet some process in the first muscle stimulated the nerve laid upon it and caused the muscle in the demonstration preparation to manifest “secondary contraction.” Consider the classes of stimuli already studied and give reasons for any surmise as to the nature of the stimulus that affected the nerve of the second muscle. The tetanizing current produced in the first muscle a continuous contraction; does the evidence in this experiment indicate whether the process in the first muscle which stimulated the nerve of the second was continuous or intermittent?

Stimulation by the Current of Injury. — With care not to injure the neighboring tissues unnecessarily, dissect out the frog’s sciatic nerve from the spinal cord to the knee, and lay the nerve on the gastrocnemius muscle. With sharp scissors cut through the muscles of the thigh. Lift the nerve with a glass rod, lay the trunk of the nerve against the cut ends of the muscles, and bring the cut end of the nerve in contact with the uninjured surface of these muscles. Make and break this contact several times. What occurs at each contact of the nerve-end with the muscle? Draw a diagram showing the direction of the stimulating current. It was by such an experiment that Galvani definitely proved the existence of animal currents not due to metals.

II. SMOOTH MUSCLE

Smooth muscle is widely distributed through the body in the walls of hollow organs, such as bladder, stomach, intestines, and blood vessels. There are several important physiological characteristics of smooth muscle which distinguish it from skeletal muscle, and which bear on its functions in the hollow viscera.

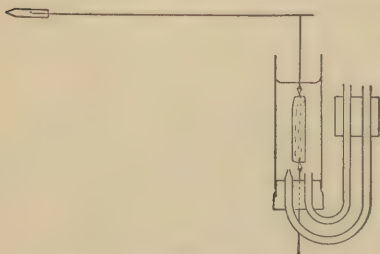


FIG. 5. — Smooth-muscle Chamber.

The following experiments are to be performed by groups of four students working together. They should prepare the chamber (see Fig. 5), and make arrangements for recording contractions.

The instructor will kill a rat, remove the stomach, intestine, and cæcum and place them in a glass dish of oxygenated mammalian Ringer’s solution. Examine the organs for muscular movements, — peristalsis and segmentation. Stimulate the surface of the cæcum electrically and observe the result. (The small intestine of the rabbit may be substituted for the intestine of the rat.)

Compare smooth and striated muscle in respect to (a) the length of the latent period and (b) the spread of contraction from a stimulated region.

Rhythmic Contractions. — With a light aluminum lever record the movements of a short strip of intestine suspended between threads in Ringer's fluid. The writing point should be long and flexible and should bear gently upon the surface of a *lightly* smoked and *slowly* revolving drum. Oxygen must be bubbled through the chamber slowly but continuously. After the preparation is set up, a temperature of 35–37° C. should be established in the chamber with the aid of a water bath.

Note the character of the movements, their amplitude and frequency.

Compare smooth and striated muscle in respect to (a) the occurrence of spontaneous movements, (b) the speed of contraction, and (c) the strength of contraction.

The Innervation of Smooth Muscle. — The reaction of a tissue to adrenin may be taken as evidence that it is innervated by sympathetic nerves (Elliott, J. Physiol., 1905, xxxii, 401). Record the result of surrounding the strip with adrenalin chloride, 1 part in 100,000 of Ringer's solution, at 37° C.

Wash out the adrenalin solution.

Is smooth muscle dependent on its extrinsic innervation for movement or tone? Compare with skeletal muscle.

What is the effect of sympathetic nerves on the tone and rhythmic contractions of intestinal smooth muscle?

The Influence of Tension. — Increase the load on the muscle by hanging very light weights at different distances from the fulcrum of the lever.

What is the effect on tone and rhythm?

The Influence of Asphyxia. — Shut off the flow of oxygen and record the result. Renew the flow as soon as an effect of oxygen-lack is seen.

What is the effect of asphyxia on tone and rhythm?

The Influence of Temperature. — Replace the water bath with a beaker of ice water. When the solution surrounding the muscle has become thoroughly chilled, make on a fresh drum a record of the effect of raising the temperature *slowly* to 60° by warming the water in the beaker. The temperature *inside the chamber* should be noted from time to time.

What is the effect of temperature changes on rhythm and tone?

Compare the behaviour of smooth and skeletal muscle on being heated to temperatures incompatible with life. (See Meigs, Am. J. Physiol., 1909, xxiv, 1).

III. *CILIA

One of the primitive forms of contractility to be found in higher organisms is ciliary movement. The ciliated surfaces of various tubular structures of the body have important functions to perform and are capable of doing a surprising amount of work. The manner in which these ciliated surfaces perform their functions can be observed in the frog.

The Functioning of a Ciliated Surface. — Pith the brain and cord of a frog and fasten the animal back downward on a frog-board. Remove the ventral body wall and all of the

viscera except the oesophagus and stomach. With heavy scissors cut through the middle of the lower jaw and continue the cut to the stomach. Draw back the flaps of the lower jaw and pin out the oesophagus to form a flat surface level with the roof of the mouth.

Place a small piece of cork on the mucous membrane covering the roof of the mouth. In which direction does the cork move?

Tilt the frog-board so that the cork would be carried up an incline. Is the cork raised by the beating of the cilia?

Lay the frog flat again and determine the time required for the cork to move 2 centimetres. Apply to the mucous surface isotonic salt solution warmed to 30°. Again determine the rate of movement of the cork. How is ciliary activity affected by warming?

Hold above the mucous surface a piece of filter paper which has been dipped in ether, and blow the vapor down upon the preparation. After a few seconds make another determination of the rate of movement of the cork. What is the effect of ether vapor on ciliary activity?

Might the effect be in part due to cooling?

The Movement of a Single Cilium. — Open the shell of a clam, and catch in a beaker the contained fluid. Cut from the edge of one of the gills a small piece of tissue; tease it apart gently with needles and place it on a microscope slide in some of the fluid from the clam. Set a cover glass over the preparation. As time elapses the cilia beat more slowly. Examine the preparation from time to time with the high power of a microscope; examples of active cilia, of inactive cilia, and of slowly beating cilia will be found. Make a series of diagrams showing successive phases of the movement of a single cilium.

CONDUCTION IN THE NERVE TRUNK AND IN THE NERVOUS SYSTEM

I. THE NERVE TRUNK

THE study of the phenomena of contraction has revealed the characteristic activities of skeletal and smooth muscle and the manner in which certain external conditions may affect these activities. Muscles are the end organs of nerves, however, and without nervous control are functionally useless. It is necessary, therefore, in order to understand the orderly interaction of muscles in the body, to study them with reference to the nervous system.

The peculiar function of the nervous system, as already noted, is the conducting of impulses from one point to another. This simple function is modified by augmentation or inhibition, and in other ways, when more than one neuron is involved in the conducting path, as, for example, in the spinal cord and brain. A study of the phenomenon of conduction in its simplest form in the peripheral nerve trunk will be the best approach to the more complicated conditions prevailing in the central nervous system.

The Insulation of Nervous Conduction.—Eviscerate a pithed frog and remove the skin from the hind limbs. Lay the limbs on a clean glass plate with the sacral plexus exposed. By means of a wire connect the tissues near the spinal column with the earth through a gas or water pipe. Attach to another wire a pithing needle, and fasten the wire to one post of the secondary coil. With the induction coil arranged for minimal tetanizing currents, touch the sacral nerves at different points with the needle electrode, and observe local contractions in the sartorius or other muscles, according to the nerve fibre touched by the needle.

The sartorius is normally innervated so that it contracts as a single unit. In this experiment the stimulation of certain nerve fibres has caused only partial contraction of the muscle. The presence of nerve impulses in fibres leading to the contracting portion has not aroused impulses in fibres leading to the portion which did not contract. What conclusion may be drawn as to the influence of nerve impulses on contiguous inactive nerve fibres?

The method of stimulating used in this experiment is that of *unipolar induction*. When a current is made and broken in a primary coil, the wires of the secondary, if in open circuit, have developed upon them an electrostatic charge. If this becomes sufficiently great, there is a discharge through any conducting path connecting the coil with the earth. If irritable tissues lie in this path, they are stimulated. In any experiment in which currents of high potential are used as stimulating agents the possibility of unipolar induction should be kept in mind, for it is likely to lead to mistakes in observation.

***The Nerve Trunk may conduct in Both Directions.**—Pith the brain and spinal cord of a frog. Carefully sever one of the gracilis muscles at its insertion and turn it to reveal its inner surface. An examination of this surface of the muscle shows that its nerve branches; one branch runs toward the hip for some distance without dividing, the other, running

toward the knee, almost immediately divides in the substance of the muscle. Corresponding with this branching there is a branching of individual axons.

Careful examination will show that the muscle is divided into a larger and smaller portion by a tendinous intersection. With sharp scissors divide the muscle transversely along the tendon, in the region of the undivided branch which runs toward the hip. Take great care not to injure the nerve, which should be the only connection between the two parts of the muscle.

Pinch the lower twigs of the nerve with small forceps. What happens in the two parts of the muscle?

In what direction does the nerve impulse normally come to the lower portion of the muscle?

In what direction must the impulse have gone in this experiment in order to stimulate the upper portion?

What conclusion may be drawn as to the ability of the nerve to conduct in both directions?

The Rate of Conduction in the Nerve Trunk. — Observations on muscle showed that the contraction spreads as a wave from the point of stimulation over the rest of the stimulated fibres. Examination by means of an electrometer proves that the nerve impulse also sweeps along the nerve as a wave. The rate of nervous conduction was formerly believed to be incalculably fast, but Helmholtz proved not only that the speed of the nerve impulse could be determined but that its rate was not extraordinarily rapid (*Arch. f. Anat., Physiol. u. wiss. Med.*, 1850, p. 328). The method he used was similar to that described in the following experiment.

In the primary circuit of an inductorium include both a key and an electromagnetic signal. Clamp the signal to an iron stand so that its record is written directly and closely below that of the muscle lever. Fix above the muscle lever a moist chamber with a large piece of moistened filter paper adherent to the inside of the cover. In two spring clips on the horizontal bar in the moist chamber set two pairs of needle electrodes. The points of each pair of needles should be only a millimetre apart. Using the pole-changer as a double key, attach the middle posts to the secondary coil and connect each pair of end posts with a pair of needle electrodes.

Make from a *large* frog a preparation of the gastrocnemius muscle with the full length of the sciatic nerve. Fasten the femur in place in the moist chamber; bring one pair of needle electrodes as close as possible to the knee joint, set the other pair as far away on the rod as the nerve will reach, and lay the nerve in contact with all four needle tips. Measure the length of the nerve between the pairs of electrodes. Arrange the muscle for graphic record, and load it only with the weight of the lever. Since only the beginning of the contraction is of interest, the axis of the muscle lever may be tilted so that as the writing point rises it will cease recording.

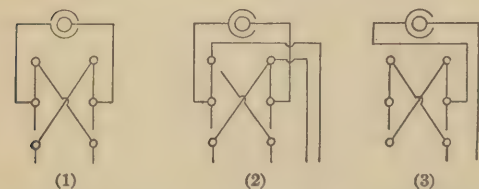


FIG. 6. — Diagrams showing different uses of the pole-changer. (1) To reverse the direction of the current. (2) With cross wires disconnected, as a double key. (3) As a simple key. In the metal contact pole-changer the cross wires are disconnected by slightly unscrewing the nuts which make the contact between the wires and the blocks through which they pass.

With the tuning fork vibrating, spin the drum very rapidly, and stimulate the nerve through the electrodes distant from the muscle with a maximal make induction shock. Next secure a record when the nerve is stimulated near the muscle. Obtain six pairs of records. In each

instance be careful to prepare for a subsequent determination of the latent period of the contraction (see p. 21).

After the records have been made permanent, calculate the latent periods for near and for far stimulation. What is the average for each set of observations?

What is the greatest variation from that average?

What is the difference between the average figures?

To what is this difference due?

Calculate the rate of the nerve impulse per second.

***The Influence of Some Mechanical Agents.** — Shaking, pinching, striking, and stretching have been used as mechanical agents to arouse nerve impulses and to modify their passage along nerves. In an earlier experiment the effect of pinching a nerve has already been seen. The stretching of a nerve may be used to modify conduction.

Determine the minimal induction shock for the nerve of a nerve-muscle preparation. Make a small paper cone, tie it to the end of the nerve, and place in it a one-gram weight. Stimulate with the same current as before. If the muscle responds, add more weights to the pan, and after each addition test the conduction by repeating the stimulus.

At what tension did the nerve fail to conduct?

Was there at any time evidence of increased irritability?

***The Influence of Some Chemical Agents, Ether and Chloroform.** — Make a nerve-muscle preparation with a long sciatic nerve and clamp the femur in the moist chamber. Set the gas chamber upside down in the moist chamber. Draw the nerve through the two small holes in the gas chamber and fill the remaining space in the holes with kaolin moist with isotonic salt solution. Record the response to a minimal single stimulus when the electrodes are applied to the end of the nerve.

Dip a piece of filter paper in ether and place it in the chamber. At regular intervals test the conductivity of the nerve by applying the minimal stimulus. Note the time before the conductivity is abolished. Remove the gas chamber with the filter paper and blow away the ether vapor. Test the nerve at regular intervals for a return of conductivity.

Repeat the experiment with another nerve, but use chloroform instead of ether. Compare ether and chloroform as to the time required for their effects to appear and disappear.

II. THE CENTRAL NERVOUS SYSTEM

Thus far the study of conduction in nervous tissue has been restricted to the relatively simple conditions presented by the nerve trunk. When impulses pass the limit of a single neuron, and two or more cells are involved in the conducting path, the conduction is at once manifestly different from that in the outlying nerve fibres. In all probability each new cell must be roused to activity before it can transmit the impulse it receives, and it is probable also that each cell modifies the impulses which it transmits. In other words, the cells are biological units and not merely passive conductors. The peculiarities of conduction involving more than one neuron are revealed in observations on the functioning of the central nervous system. In the central system the primitive functions are the reception of impulses from the body surface and from the interior of the body, and the sending of impulses to outlying muscles and glands. These primitive functions are illustrated in the simple reflex.

Reflex actions are usually not vigorous. Do not attempt to evoke them when they are hindered by the friction of the body against a supporting surface. Always suspend the animal to be studied with the limbs free to move.

Spinal Shock.—Pith the brain of a *large* frog. In doing so point the pithing needle slightly backward at the close of the operation in order to be sure to destroy the medulla. Immediately suspend the animal from a pin hook passed under the lower jaw. Stimulate the toes of one foot with a tetanizing current. What is the result?

Place the frog in a basin of water. Compare its position with that of a normal animal.

Wait a few minutes and repeat these observations. What now is the result of stimulation? Is there any difference in the attitude of the animal in the water?

The depression of function following severance of a part of the spinal cord from the rest of the central nervous system is known as spinal shock. The shock is much more marked in the part separated from the brain. In the frog the phenomenon soon disappears, but in some mammals it may last for weeks before the reflexes are recovered. Compare this feature of the functioning of the central nervous system with conduction in the nerve trunk.

Reflexes involve the Central Nervous System.—Suspend by the jaw the frog with brain pithed and pinch one foot sharply with forceps. Note the resulting movement. When did the movement occur, at the moment of stimulation or distinctly some time after stimulation had ceased? Compare this result with the result of mechanical stimulation of the nerve trunk.

Apply the same stimulus to one of the fore limbs. If there is a reflex response, pass a pithing wire into the spinal canal and cautiously destroy that part of the upper cord connected with brachial nerves. Do not destroy more than this section of the cord. Can reflexes from the fore limb still be evoked?

Does the evidence indicate that afferent impulses must reach the central system before producing reflex effects?

The Functions of the Dorsal and Ventral Roots.—Prove that the reflexes of the hind limbs of the frog used in the foregoing experiment are still active. Remove the skin from the back between the tip of the urostyle and the region of the fore limbs. Cut away the muscles overlying the vertebrae. Cautiously isolate the urostyle from its muscular attachments, without injury to the sciatic plexus lying below. Disconnect the bone at the joint. The spinal canal is now exposed as a rounded opening in the ninth vertebra. Into the canal insert merely the tip of the sharp-pointed blade of heavy scissors, and cut the dorsal laminae first on one side and then on the other in successive vertebrae towards the head. At each step take great care not to permit the scissors to press upon the spinal cord. After the first two or three vertebrae have been thus cut the continuance of the operation is facilitated by lifting the separated part of the canal with forceps. In this manner the entire central nervous system may be uncovered.

The *filum terminale* is a dark gray thread lying along the ventral wall of the vertebral canal. With small forceps cautiously raise it out of the canal and draw it to one side. As this is done the dorsal roots of the spinal nerves first come clearly into view and under them the ventral roots. Note that they differ in size. Moisten a fine silk thread and tie it around the middle of a dorsal root. About a millimetre from the first ligature tie another.

Sever the nerve between the ligatures. Unless the threads are manipulated with the greatest care the nerves will be pulled away from the cord.

In stimulating these nerves the unipolar method (see p. 26) gives the advantage of narrowly localized stimulation. Attach by a wire the flat brass electrode to one post of the secondary coil. Wind the end of another wire around the end of a pithing needle, and thus connect the needle with the remaining post of the secondary. In this experiment the frog lies on his belly. Place the indifferent electrode under the frog. Determine the weakest tetanizing current which when applied to the central part of the dorsal root will cause a reflex movement of muscles. Apply the same stimulus to the distal part of the dorsal root. What is the result? What may be inferred as to the function of the dorsal root?

Similarly double-ligate a ventral root and cut between the ligatures. Determine the weakest tetanizing current which, when applied to the distal part of the root, will cause a contraction. Apply the same stimulus to the central part of the ventral root. What is the result? What may be inferred as to the function of the ventral root? (Bell, *New Anatomy of the Brain*, 1811; Magendie, *J. physiol. expér.*, i, ii, iii, 1821-23.)

Forward Conduction. — Lay bare the sciatic nerve in the thigh and sever it near the knee. Suspend the frog by a hook. Apply a strong stimulus to the nerve central to the cut, and secure a crossed reflex, *i. e.*, a reflex from the opposite leg. Cut all posterior roots connected with the cut sciatic nerve. Again suspend the frog, and stimulate the nerve central to the cut. Is it possible to cause a reflex response?

Within the central nervous system there are evidently connections between afferent and efferent conducting paths. A stimulus applied to a root bearing afferent impulses will cause a response not only in the nearest efferent path, but by way of other efferent nerves. In this experiment stimulation of the sciatic nerve necessarily starts impulses which pass along its whole length.

Is there any evidence that these impulses in the motor fibres get beyond the limits of the motor neurons?

What inference may be drawn as to reversibility of conduction in the reflex arc?

Compare conduction in the reflex arc with conduction in the nerve trunk, cf. p. 33 (Johannes Müller, *Handb. d. Physiol. d. Menschen*, Coblenz, 1838, i, 688).

The Segmental Arrangement of Reflexes. — There is both morphological and physiological evidence that primitively the nervous system is composed of a series of like parts along the body axis. The repetition of spinal nerves at regular intervals on the spinal cord is significant of the primitive condition. Reflex responses from portions of the cord also indicate a segmental arrangement of reflex mechanisms, though they may not be confined to single morphological segments.

The Reflex corresponds to the Region Stimulated. — Pith the brain of a frog and suspend the animal from a hook. Gently pinch the toes of the right foot. Where is the response?

Repeat with the left foot. Where is the response?

Pinch a fore limb. Where is the response?

The Spreading of Reflexes in the Cord. — Stimulate with a weak tetanizing current the right toes of a suspended frog whose brain has been pithed. Repeat the stimulus, after making it stronger. The weak stimulus is likely to result in a reflex directed to the side

stimulated. The stronger stimulus is likely to result in a symmetrical crossed reflex. On which side is the response more vigorous?

The stronger stimulus tends also to cause reflexes in other segments nearer the head. What does this variation in the ease of evoking reflexes indicate as to the resistance to the spreading of impulses in the central nervous system?

What is the easiest course for an afferent impulse to take? What the next easiest?

Compare with conduction in the nerve trunk this graded resistance when more than one neuron is involved in the conducting path (Pflüger, *Die sensorische Functionen d. Rückenmarkes*, Berlin, 1853, p. 67).

The Effect of Strychnine.—Introduce into the dorsal lymph sac of the frog used in the foregoing experiment a few drops of a 0.5 per cent solution of strychnine sulphate. Suspend the frog and, after a short period, apply a weak stimulus to any part of the body. What is the character of the contraction?

When the contraction ceases apply the stimulus to another part of the body. Repeat the stimulation at different points whenever contraction ceases. What inference may be drawn as to the influence of strychnine on the graded resistances in the central nervous system?

**Response from Pieces of the Spinal Cord.*—Eviscerate a frog with brain pithed, sever the cord back of the brachial nerves by pressing a sharp knife between two adjacent vertebrae. Do reflexes from the fore limbs still persist?

Do reflexes from the hind limbs still persist?

If there are still reflexes from the hind limbs, transect the cord at the vertebral joint next posterior to that already transected. Continue thus until the reflexes from the hind limbs cease.

The central nervous system is evidently capable of performing its elementary function in small segments. The results secured in the strychninized frog when the stimulus was applied to various points on the body surface clearly prove, however, that the separable reflexes of the several segments are normally closely joined and that the system is a unity.

(Demonstration.—Reflexes in a Spinal Mammal.—While a cat is anaesthetized with ether, the common carotids are tied, a cannula is fastened into the trachea, and arrangements are made for artificial respiration. With scissors a small opening is made in the skin over the atlas. By means of a pithing needle introduced through this opening the cord is completely transected and the brain destroyed. At once artificial respiration without anaesthesia is started. The trunk of the animal is now suspended horizontally with the legs hanging free. Care is taken to maintain the body temperature. After a short period this spinal preparation will manifest many complicated reflexes involving both long and short conducting paths in the cord,—among them the scratch reflex on tickling the neck behind the ear, the extensor thrust on pressing upward with the hand against the bottom of a hind foot, and the flexor reflex on pressing between the pads of the foot with a sharp-pointed instrument. This demonstration can be made also on a decapitated animal in accordance with the original directions. (See Sherrington, *J. Physiol.*, 1909, xxxviii, p. 375.))

Muscular Tonus.—Muscular tonus or tone is a state of slight contraction in which muscles are maintained while connected with the central nervous system. It is most marked in muscles which serve to keep the natural posture of the body.

Pith a frog's brain, and by a dorsal incision expose the sciatic plexus on one side. Cut all the sciatic nerve components on that side. Suspend the frog by a hook. On which side do the toes hang lower?

Is there any difference in the appearance of the joints at the two sides?

Let the frog hang in a battery jar nearly filled with water. Does the difference between the two legs increase? (Brondgeest, Onderz. over d. Tonus d. willekeur. Spieren, Utrecht, 1860.)

Decerebrate Rigidity and the Reciprocal Innervation of Antagonistic Muscles in the Cat. — When in a decerebrate or spinal cat or dog an afferent nerve of the hind limb is stimulated, the normal dominant reflex responses are as follows:

1. The flexion reflex, consisting in (a) excitation of the flexor muscles and (b) inhibition of the extensor muscles in the same limb as the stimulated nerve.
2. The crossed extension reflex, consisting in (c) excitation of the extensors and (d) inhibition of the flexors in the opposite hind limb.

The following experiment is intended to demonstrate decerebrate rigidity and three of the four reflex responses — (a), (b) and (c). The effects may be shown by applying the stimulus directly to a nerve trunk or by stimulating with sufficient intensity the skin at the extremity of a limb. All the main nerve trunks of the limb, including the branches ending in muscles, contain afferent fibers. The central end of any large nerve severed in the limb will serve, therefore, to evoke a reflex response.

Decerebrate Rigidity. — A cat is etherized and ligatures are tied around both carotid arteries. The cranium is trephined over one hemisphere and the opening enlarged with rongeur forceps. The cerebral hemispheres are then removed at the level of the anterior colliculi without injury to other parts of the central nervous system. Or the decerebration may be done by means of a Sherrington decerebrator (see Sherrington, J. Physiol., 1915, xlix, p. lii). During the operation for removal of the cerebrum, the vertebral arteries should be compressed by gripping the animal's neck just behind the transverse processes of the atlas. As soon as the cerebrum is destroyed the anaesthetic is discontinued. The limbs in a short time become rigidly extended. The rigidity is due to an increased reflex tone in the extensor muscles, *i. e.*, the muscles which in the normal condition keep the animal supported against the pull of gravity. The rigidity can be overcome by severance of the afferent roots from the muscles, or by afferent impulses from the skin. To test this possibility a beaker of hot water may be raised about one of the fore feet or one of the hind feet, and the result noted. (Sherrington, J. Physiol., 1897, xxii, 319; *The Integrative Action of the Nervous System*, New York, 1906, pp. 299–305.)

The Reciprocal Action of Antagonistic Muscles. — Make an incision in the lateral aspect of the right hind leg from hip to knee, dissect back the biceps femoris muscle, and expose the sciatic nerve in this region. Tie a ligature around the nerve as far down in the popliteal space as possible and cut distal to the ligature. It is advisable to have an assistant compress the vertebral arteries during this operation, since the resulting rise in blood pressure may start a fresh hemorrhage in the brain stem. Apply a pair of Sherrington shielded electrodes to the central portion of the cut sciatic nerve, being careful to avoid stretching or jamming the nerve trunk in the process. The electrodes are connected with the secondary coil of an inductorium arranged for single make and break shocks. Stimulate the nerve, first with single shocks, allowing several seconds to elapse between stimulations. Observe the reflex flexion of hip and knee joints, and extension in the opposite leg. Stimulate with

a series of make and break shocks at a frequency of four or five a second and note the after-discharge and resulting cumulative effect of a series of stimuli in these reflexes.

Having made these observations, prepare the limb for demonstration of the inhibition of extensor muscles that plays a part in the flexion reflex. For this purpose the knee joint will serve as an indicator. All flexor muscles must be prevented from acting on it, lest their action obscure the relaxation of the extensors. Remove the stimulating electrodes from the sciatic nerve, cover it with the muscle, and close the wound. Cut the nerve to the hamstring muscle, that branches off from the sciatic nerve at the hip. Make an incision from the groin half way to the knee on the inner aspect of the right leg, expose the branches of the anterior crural nerve, sever the branch leading to the sartorius muscle and, if you are able to isolate it, also the branch innervating the rectus femoris muscle, thus leaving the vasto-crureus group, which are pure extensors, as the only innervated muscles acting on the knee joint. Close this wound with sutures. Again open the lateral wound. Clamp the femur in a vertical position with the knee upward. There will probably be enough tonic contraction of the vasto-crureus muscle to maintain the knee in a state of partial extension. Flexion of the knee cannot now result from active muscular contraction, for all flexors have been paralyzed by section of their motor nerves. Relaxation of the vasto-crureus, however, will result in flexion of the knee due to the weight of the lower limb and foot. Flexion, therefore, can only mean inhibition or relaxation of extensor muscles. Reapply the shielded electrodes to the sciatic nerve and by means of a suture passed through the adjacent tissues and tied tightly around the neck of the shield secure it in such a position that the nerve will not be jammed or compressed. Pass a long suture through the skin over the shin about an inch distal to the knee joint, and tie it to a muscle lever placed directly over the knee joint.

From a rod secured higher up on the stand support the weight of the muscle lever with a long rubber band of sufficient strength to keep the thread between the lever and the leg barely taut. Contraction of the knee extensors will then cause the lever to rise, and relaxation will cause it to fall. Prepare a smoked drum to record the motions of the lever. Arrange the primary circuit for a tetanizing current and introduce a signal magnet.

With the drum moving about 1 mm. a second stimulate with a moderate strength of induction shocks for two or three seconds. If the knee extensors are in a state of tonic contraction, central inhibition will be clearly revealed by their relaxation and the resulting fall of the lever. Following cessation of the stimulus there may be a "rebound" contraction, greater in extent than the pre-existing "tonus." This reaction is not always found. If there is no tonic contraction, and inhibition therefore fails to appear, pinch the toe pad of the *left* hind leg with sharp forceps. This will usually evoke a crossed extension reflex which may then be inhibited by stimulation of the right sciatic nerve. If this procedure fails to evoke a satisfactory crossed extension reflex, and if time permits, expose and sever the left sciatic nerve, and stimulate the central end with a second inductorium. While the resulting crossed extension reflex is in progress, stimulate the right sciatic nerve as before, and record the inhibition of extensors. (See Sherrington, *The Integrative Action of the Nervous System*, New York, 1906, pp. 90-100.)

The Reciprocal Innervation of Antagonistic Muscles in Man.—Let the subject gently flex one arm, and let him hold the hand, palm forward, approximately on a level with the shoulder. Feel the biceps muscle of the flexed arm; it will be slightly hardened in contraction. While continuing to feel the muscle, ask the subject to move so as to press the hand against an immovable object—a wall or a door casing. As the pressure is applied the triceps contracts strongly. What change occurs in the "feel" of the biceps?

While he is pressing strongly, ask him to try simultaneously to contract the biceps. Does the muscle harden ?

These observations may be made on one's self.

Effects of Related Stimulations. — When simultaneous or immediately successive excitations are produced, one of two results may occur. There may be a summation of the two excitations — a reinforcement of one by the other, or there may be an inhibition of one excitation by the other.

Summation. — Suspend by a hook a frog with brain pithed. Tie two fine copper wires a centimetre apart around the left foot, near the toes, and attach the wires to the secondary coil of an inductorium. Connect the primary coil through a simple key with a dry cell. Do single make and break shocks evoke a reflex response ?

Be very careful in this case to distinguish between direct stimulation of the muscle by the electric current and reflex stimulation from the central nervous system.

Stimulate with regularly repeated weak make and break shocks, and test whether under these circumstances reflex action can result from the summation of afferent impulses.

If the stimuli are repeated more rapidly, does the reflex occur sooner ?

What is the effect of increasing the strength of the stimuli and maintaining the same rate of stimulation ?

Inhibition. — Use the frog and the apparatus as described in the foregoing experiment but arrange the inductorium to deliver a tetanizing current.

Provide a vessel of water. Immerse the toes of the right leg of the frog in 0.5 per cent sulphuric acid and note the time required before reflex flexion occurs. Without any delay immerse the leg in the water and wash off the acid.

After an interval of three minutes, stimulate the left foot with a weak tetanizing current as the right foot is again immersed in the acid. If the foot is not withdrawn from the acid after 20 seconds have elapsed, stop the tetanizing current and note the results. What has been the effect of the afferent impulses from the left foot on the efferent impulses to the right leg ?

After again washing the leg in water, prove that the sensory endings in the skin are still irritable to the acid.

Spinal reflexes are characteristically inhibited by impulses from the brain. With sharp scissors quickly sever the skull of a frog immediately in front of the tympanic membrane — an operation which removes the hemispheres but leaves the optic lobes. Determine by means of the foot dipped in weak acid the reflex time. After washing off the acid, apply crystals of sodium chloride to the exposed optic lobes, and again determine the reflex time. Is the period prolonged ?

Augmentation of the Knee-jerk in Man. — Cause the subject to sit with the knee flexed at nearly a right angle and with the foot hanging free. It is essential that the muscles be relaxed. This condition can sometimes be best secured by letting the foot rest on a board supported by rollers. Two round pencils will serve as rollers. Place one index finger over the patellar ligament and strike the finger a sharp blow. What movement of the foot results ?

Let the subject pull upon his clasped hands with a maximum muscular effort, and while he is doing so, again strike the finger, laid over his patellar ligaments, the same sharp blow as before. Is there any notable increase in the activity of the knee-jerk?

Instead of clasping the hands, the subject may extend the toes of the leg to be treated. Is there evidence of "reinforcement"?

The knee-jerk is possibly not a true reflex, for the response perhaps occurs too soon after the stimulation to permit an impulse to pass to the cord and back at the usual rate. The response accompanies, however, the existence of a state of tone in the extensor muscles, and since this is dependent on the integrity of the reflex arc, the presence of the knee-jerk is significant of a normal condition.

Inhibition of a Reflex in Man. — When about to sneeze, press strongly against the upper lip just below the nose. What is the effect?

Observations on Reflexes in Man. — The existence of a normal reflex demonstrates the integrity of the afferent and efferent nerves and the segment of the brain or spinal cord involved in the act. The absence of the reflex shows that the path is interrupted. Increased activity of certain reflexes indicates destruction of superior neurons. The study of human reflexes, therefore, is important in determining the seat of injury to the nervous system. The utility of the reflexes in this respect can be well illustrated in any large neurological clinic.

The following examples will show the methods employed in testing the integrity of reflex arcs in man. In each instance the student should state, so far as his anatomical knowledge serves, the in-going and out-going paths and the segment of the central nervous system concerned.

(1) **The Sneezing Reflex.** — Tickle the interior of the nostril with a thread. State the muscles and nerves involved in the reflex.

(2) **Conjunctival and Corneal Reflexes.** — Touch the cornea with a thread or a twisted bit of absorbent cotton. Be careful to avoid touching the eyelashes. Record the response.

(3) **Pupillary reflexes.** — Cause the subject to face a window so that there will be equal lighting of both eyes. Screen the eyes with the hands. After several seconds expose the eyes to the light. What changes occur in the pupils?

Screen one eye. Watch the other pupil when the screened eye is exposed to the light. Does the pupil change?

Turn the subject away from the light and have him look alternately at a near and at a far object without change of the lighting conditions. Describe the alteration in the size of the pupil. In converging the eyes for seeing a near object, what muscles are employed? What is their innervation?

The preservation of the accommodation reflex with loss of the reaction to light is the condition known as "The Argyll-Robertson pupil."

(4) **Cilio-spinal Reflex.** — Pinch the skin on the nape of the neck while watching the pupils. Record the change in the size of the pupils.

(5) **Pharyngeal Reflex.** — With a blunt instrument touch the back of the pharynx. State all the responses.

(6) **Abdominal Reflex.** — With the finger nail or a small rough object like the end of a match, administer a quick sharp stroke just below the line of the ribs. What is the effect?

(7) The Cremasteric Reflex. — Make a long scratching stroke on the inner side of the thigh. Note the reflex contraction of the cremasteric muscle.

(8) The Achilles Jerk. — Cause the subject to kneel on a chair or stool. Bend his foot at right angles to the leg and gently press upward the ball of the foot to increase the tension of the gastrocnemius muscle. Strike the Achilles tendon with the side of the hand.

The response in this case is similar to the response of the extensors of the thigh, and can be felt in the muscles of the calf. It occurs as well if the tendon is struck on the side, but does not occur if, under these circumstances, the tendon is so supported that the side blow does not stretch the muscle.

(9) The Plantar Reflex. — Tickle or scratch the sole of the subject's foot. Describe the reflex response of the toes.

The Nature of Purposive Reflex Action. — Suspend from a hook a frog with brain pithed. Dip in acetic acid a small piece of filter paper about a half centimetre square. Shake off the excess of acid, then apply the paper to the front of the frog's body. What movements result?

Remove the paper, dip the frog in water to wash the acid from his skin, and again suspend the animal from the hook. After five minutes repeat the experiment, but apply the acidulated paper to the inner side of the thigh. If only one foot is drawn up hold that foot. Does the other foot now move?

After washing off the acid and waiting again for five minutes, apply the acidulated paper to the back near the tip of the urostyle. To what region is the response now directed?

Are the directions of the reflex movements sufficiently different in these three instances, and pointed toward a definite end with sufficient clearness, to indicate purposive action?

Purposive movements are not necessarily intended movements. It is probable that reflex action directed with apparent purposefulness is in reality an automatic repetition of movements developed for certain effects in the previous experience of the intact animal. The nature of the spinal animal may be judged from the following experiment.

Place in an evaporating dish lined with cloth or cotton and containing enough water to permit the animals to float, a normal frog and the spinal frog which reacted to the acid stimuli. By means of a flame warm the water.

As the water is warmed what is the reaction of the frog with the brain intact? Set this frog aside.

What is the reaction of the spinal frog? Explain the result as the temperature of the water rises above 40°C.

What inference may be drawn as to the reactions to the acid stimuli; were they purposed, or merely purposive? (Goltz, *Beitr. z. Lehre v. d. Funct. d. Nervencentren d. Frosches*, Berlin, 1869, p. 127.)

The Activities of the Decerebrate Frog. — The reflexes hitherto studied have been relatively simple in character. More complicated reflexes, involving the coördination of more extensive movements than those of a single limb, are observed when, in addition to the spinal cord, there are retained as seats of reflex response, the medulla, the cerebellum, and the optic lobes. The effects of removing the cerebral hemispheres in different animals vary with the complexities of the hemispheres and the degree of their control over the movements of the organism. In higher vertebrates the removal causes profound alteration of the reactions to surroundings. The effects of decerebration on one of the lower vertebrates are seen in the following observations on the hemisphereless frog.

The Removal of the Hemispheres.—Select a male frog, characterized by a thickened pad on the innermost digit of the front limb. Anaesthetize him by placing him under a battery jar with some absorbent cotton wet with ether. If during the following operation the effect of the anaesthetic diminishes, replace him under the jar.

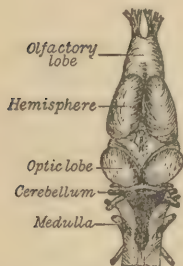


FIG. 7.—Brain of frog, dorsal aspect (after Gaupp).

The cerebral hemispheres of the frog extend back to a line connecting the front margins of the two tympanic membranes. Cut the skin along this line over the top of the skull. From this cross-cut make a median incision forward nearly to the nostrils. Lay back the flaps. With scissor points separated to either side of the top of the skull, immediately in front of the transverse skin incision, cautiously bring the points together, cutting barely through the bone. Insert the sharp blade forward and at one side under the bony covering of the cerebrum, and snip the bone. Repeat the operation on the other side. Raise the bone with small forceps and carefully cut forward, alternately on the one side and the other, until the cerebrum is entirely exposed. Sever the connections between the optic lobes and the cerebrum, and remove it. With silk thread sew together the flaps of skin.

Note the posture of the animal immediately after the operation. To what factors may this be due? Pith the brain of another frog. While the animals are recovering, perform other experiments. During the interval, however, keep the skin moist, for the frog breathes in large part through his moist skin. In about an hour test the capabilities of the frogs as follows.

(1) Posture.—Record the difference between the decerebrate frog and the spinal frog as to posture.

(2) Locomotion.—Similarly record the differences in leaping and swimming.

(3) Respiration.—Is there a difference in respiratory activity between the decerebrate and the spinal animal?

(4) Vision.—Compare the eyelids in the decerebrate frog with those in the spinal frog.

Place an opaque object between the decerebrate frog and a source of light. With the animal facing the object, which should be only six or seven centimetres distant, stimulate him to jump. Does the frog jump against the object or avoid it? (Goltz, loc. cit., p. 65.)

(5) Equilibration.—Turn the decerebrate frog on his back. Compare his reaction with that of a spinal frog. Place him on the palm of the hand or on the frog-board. Slowly tilt his support. What happens as his equilibrium is disturbed? Continue the experiment to see if the frog can be made to crawl to the other side of the hand or the frog-board, as the support is further turned.

(6) Croak Reflex.—Hold the decerebrate frog gently between the thumb and first finger, placed immediately behind the fore limbs. Apply slight pressure for a moment. The frog should croak in response to each application of pressure. Stroke with the moistened finger the skin of the back or flanks and note whether this stimulus likewise evokes the reflex (Goltz, loc. cit., p. 3).

(7) Verworn has reported that if a decerebrate frog is kept alive for 24 hours and the skin is then gently stroked along the sides of the vertebral column the legs will enter a state of extensor rigidity. This experiment may be performed and the course of the impulses controlling the movement determined.

(Demonstration.—The Activities of a Decerebrate Pigeon.—A pigeon is etherized, and the top of the skull is separated by means of scissors and removed. The hemispheres are now quickly excised or aspirated out of the skull cavity. The cavity is filled with cotton and

the skin-flaps sewed together. After recovery from the operation the animal's equilibrium is tested by placing him on the edge of a table, by standing him on a slowly revolving stick, and by laying him on his back. The powers of walking and flight are also observed. Note the difference between the immediate and the later effects of the operation. For the deficiencies of the decerebrate pigeon see Schrader, *Zur vergl. Physiol. d. Grosshirns*, Strassburg, 1890.)

(*Demonstration. — Stimulation of the Cerebral Cortex.* — A cat or rabbit is anaesthetized and maintained in anaesthesia throughout the following procedure and until death. The cerebral cortex is laid bare by the method used in the demonstration of decerebrate rigidity. Care is observed not to cut across the middle line, but to enlarge the opening as far forward as possible on one side. Oozing from the diploe is stopped by pressing warmed artist's wax against the edge of the bone. The dura mater is then reflected and the cortical surface exposed in the region of the crucial sulcus and immediately posterior to it. The legs of the opposite side should hang free. If now different points in the exposed region are stimulated briefly with a weak interrupted current, the motor areas for the front and hind limbs, the shoulder, the ear, and the vibrissae can be determined. Note that the results are coördinated movements. (See Fritsch u. Hitzig, *Arch. f. Anat., Physiol., u. wiss. Med.*, 1870, p. 300.))

Time Relations in Nervous Processes. — That continuous contraction of muscle is the result of repeated stimulation has already been proved. There is evidence that the ordinary sustained contraction of skeletal muscles is the result of repeated impulses discharged into the muscles from the central nervous system.

**Muscular Oscillations in Voluntary Contraction.* — Inferences regarding the rate of discharge of the physiological stimuli along nerves have been drawn from the rate of vibration of muscles in supreme contraction or in a state of fatigue.

Clamp to the table the ergograph with spring attachment, and arrange it, with a rather long straw, to make a graphic record. Place under the spring, as close as possible to the vertical support, the wrist, and with the arm at a right angle lift up with a strong effort. Note the height to which the writing point rose. Set the tuning fork at that level. Again lift upwards against the spring as before. The spring is seen to vibrate. Spin the drum and record simultaneously vibrations of the spring and vibrations of the tuning fork. At what rate does the muscle vibrate? (Schäfer, *J. Physiol.*, 1886, vii, 114.)

It is of interest to learn whether this rate corresponds with the rate of other processes in the nervous system. Speak as many of the letters of the alphabet as possible in three seconds. How many were spoken?

Think as many as possible in three seconds without speaking them. State the result.

Reflex Time. — The impulse passing along a nerve trunk has a rate which has been determined in a previous experiment. When the central nervous system is involved in the conduction, however, the impulse must pass beyond the limits of a single neuron, perhaps through many neurons, and probably at each synapse there is a delay. More time is therefore required for the impulse to traverse the reflex arc than would be required if the impulse traversed the same distance in a single nerve trunk. And when many neurons are involved in the conducting path the delay will be notably greater than if few are involved.

Connect the primary coil of an inductorium to a cell through an electromagnetic signal and a simple key. Arrange the apparatus for single induction shocks. By means of a nar-

row strip of surgeon's plaster attach a fine silk thread to the upper eyelid. With the head fixed firmly in one position, pass the thread over a pulley to a light writing lever. Let the subject touch the stimulating electrodes to the lower eyelid. With the tuning fork vibrating, spin the drum and stimulate with a maximal make induction shock. Repeat the observation several times. Calculate the reflex time for movement of the upper eyelid.

How far would a nerve impulse travel in this time at the rate determined for the frog's nerve?

About how far has the impulse travelled in this experiment?

What factors might affect the difference in rate?

Reaction Time.—Use the electrical apparatus employed in the foregoing experiment but connect another key in parallel in the primary circuit. Let the subject hold with one hand the stimulating electrodes against the tip of his tongue and with the other hand be ready to close one of the keys. With the tuning fork vibrating, spin the drum and break the primary circuit by opening the second key. As soon as the subject feels the stimulus he should instantly close his key and thus make the circuit again. Determine the interval between the stimulation and the response. Repeat the experiment several times and state the average of the results. Are there errors of observation that should be regarded?

Reaction Time with Choice.—Use the same apparatus and method as in the previous experiment, but instead of one of the simple keys insert the pole-changer, so arranged that it will close the circuit whether pressed downward in one direction or the other. The subject, who holds the stimulating electrodes against the tip of the tongue by means of the lips and teeth, and who sits with eyes shut holding the rocking key, is now to close the circuit with the left hand if he receives a strong stimulus, and with the right hand if he receives a weak stimulus. The proper position of the secondary coil to produce what will be recognized as a weak or a strong stimulus must be previously arranged. Determine the time required for the response when the subject must decide whether to use his left or his right hand. Compare this time with simple reaction time.

Neuro-Muscular Fatigue.—Already in experimentation on the central nervous system the reflexes have probably been observed to exhibit signs of weakening as they were repeated. This diminution in the vigor of the response can be explained by fatigue of the parts concerned in the reaction. It is desirable to know the relative resistance of these parts to fatigue.

The Seat of Fatigue.—Pith the brain of a frog and plug the opening with cotton to check hemorrhage. Expose the gastrocnemius muscle on the left side and the sciatic nerves on both sides. Suspend the animal by the lower jaw. Find a strength of tetanizing current which, when applied to the right sciatic, causes a reflex in the left leg. Stimulate the right sciatic with a tetanizing current until the muscles of the left leg cease contracting.

Now quickly apply the electrodes to the left sciatic nerve. Note the result.

Continue stimulating the left sciatic nerve until there is no visible effect. At once apply the electrodes to the left gastrocnemius muscle. Do the stimulated fibres contract?

Where did fatigue first occur, at the periphery, or in the central nervous system? Was there primary fatigue in the nerve trunk? Did the fatigue occur primarily in the muscle? What other seat of fatigue than those mentioned might explain the result? (Cf. Sherrington, *The Integrative Action of the Nervous System*, New York, 1906, pp. 214-223.)

Neuro-muscular Fatigue in Man.—Clamp the wooden ergograph to the table so that the pulley is beyond the edge. Let the subject place the first and third fingers of the right hand in the holes in the block, and fit over the terminal phalanx of the middle finger the leather loop of the ergograph. Attach to the other end of the chain, which passes over the pulley, an iron stand, or if that is too heavy to lift readily, a pan holding 100 10-gram weights. Tie to the chain, near the finger, a thread which runs over a wheel and is attached to a light muscle lever near the writing point. Every contraction of the finger will now raise the weight and at the same time write a record. Let the drum turn slowly. Note the time. With the hand and wrist prevented from moving, contract the middle finger on signal regularly once every second until the weight can no longer be lifted. After each contraction the weight should return to its support. As fatigue begins, be especially careful not to use other muscles to aid the muscle being tested. (See Mosso, *Fatigue*, Engl. trans., New York, 1904.)

How soon does complete fatigue occur?

Let the muscle rest five minutes and repeat the experiment. Compare the height of the contractions, the onset of fatigue, and the time for development of complete fatigue in the two cases.

Again let the muscle rest five minutes, but during that time apply massage to it. Repeat the experiment. Compare the characteristics of the contractions and the time for the appearance of the complete fatigue with the previous records.

What has been the effect of massage?

Without rest make a final curve stimulating the muscle directly by the unipolar method.

Does this curve resemble your curve of voluntary work?

Is it distinctive as compared with other curves in your room?

What do you conclude as to the seat of fatigue in these experiments?

III. THE SYMPATHETIC NERVOUS SYSTEM

***Sympathetic Innervation of the Iris.**—Pith the brain of a frog, with great care not to let the pithing needle touch the floor of the cranial cavity. Remove the lower jaw and tongue, and cut backwards a short distance on either side. Excise the mucous membrane covering the roof of the mouth and thus expose the junction of the vertebral column with the skull. On either side of the middle line is seen the levator anguli scapulae muscle passing backwards and outwards from its origin on the skull. Insert a pithing needle under a few of the fibres of this muscle near the attachment to the skull, and tear them away. Repeat this procedure until the muscle is entirely separated from the skull. A large white nerve, the vagus, is thus revealed curving outwards towards the side of

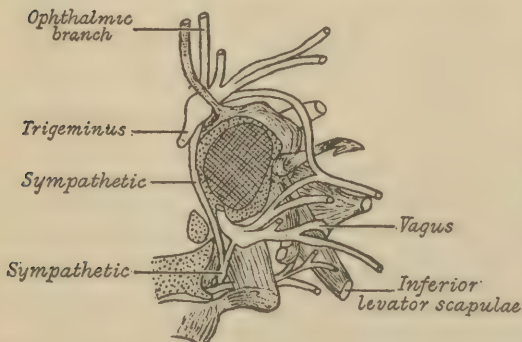


FIG. 8. — Relations of sympathetic to vagus and trigemimus nerves, dorsal aspect (after Gaupp).

the body. A grayish or yellowish nerve, lying along the vertebral column joins the vagus. This is the forward part of the frog's sympathetic trunk. It extends still further forward in the cranial cavity to the common proötic ganglion from which the 5th, 6th, and 7th cranial nerves pass off to their peripheral innervations.

Place the frog so that the eye on the operated side can be observed, and stimulate the sympathetic trunk with a weak tetanizing current. If the pupil has been small, it now becomes large. Explain the mechanism of the change.

(Demonstration. — Stimulation of the Mammalian Iris and the Smooth Muscle of Hairs. — A cat is etherized and kept anaesthetic until death. The cervical sympathetic nerve is exposed, ligatured, and severed proximal to the ligature. A weak tetanizing current is now applied to the peripheral portion of the nerve. What is the effect on the iris? How soon does the effect pass away? If in any case the pupils of the eyes are unequal, to what may the difference be due?

The laminae of the last sacral and first caudal vertebrae are removed. The gray rami in the sacral region are found drawn into the vertebral canal. A thread is tied around the cauda equina as far anterior as possible, and the nerves severed proximal to the ligature. The cauda equina is now stimulated with a strong tetanizing current. Note the change in the appearance of the tail. The sympathetic system supplies the erector pili muscles. (See Langley and Sherrington, *J. Physiol.*, 1891, xii, 285.)

THE RECEPTION OF STIMULI FOR THE NERVOUS SYSTEM

THE central nervous system receives impulses from all points on the body surface and from structures within the body; it discharges into muscles and glands. Thus changes in the environment produce reactions in the organism. Hitherto attention has been directed mainly to the reacting mechanisms, — to muscles as the “effectors” or end instruments of nerves, and to the coördination of muscular movements, through the nervous system, for the good of the organism as a whole. These movements are made, however, in response to incoming impulses. The way in which these impulses are initiated is next to be studied.

Earlier considerations showed that the agencies affecting irritable tissues could be classified into thermal, mechanical, chemical, and electrical. If photic stimuli are added, it may be said that all agents affecting living organisms, within and without, may be included in these five classes. At the outer ends of the afferent paths of the nervous system are special structures, “receptors,” specially irritable to one or another of these stimuli. Some of the receptors are within the body, as, for example, those affected by pressures of muscles and joints; others, on the surface of the body, are affected only by the contact of objects with the surface; still others, the eye, the ear, the olfactory area of the nose, receive stimuli from objects more or less remote from the body. Thus, through one receptor or another, stimuli from near and far objects in the outer world and from changes within the body itself are sifted into the nervous system as afferent impulses.

These receptors, bringing the nervous system into connection with environmental conditions, are also known as organs of special sense. Their functions are best studied in man because the states of consciousness associated with the excitation of these special organs can best be reported upon by human beings. There are general features of excitation which, if not common to the functioning of all the sense organs, are common to many of them. Such common characteristics are seen in the existence of a threshold of stimulation, a latent period, intensity, contrast, summation, fusion, inhibition, exhaustion, and after-image. It will not be possible to consider all these characteristics in relation to each receptor. In the study of one or more of the receptors, they will appear, however, in such a way as to show their significance.

A classification of the receptors according as they are affected by chemical and physical agents is to a large extent possible. But there is one group of endings of afferent nerves which can be affected by various physical and chemical agents, namely, the free nerve endings, stimulation of which causes pain. It has been pointed out that the stimuli affecting these receptors are such as tend to do immediate harm to the stimulated region. They may, therefore, be regarded as noxious stimuli.

NOXIOUS STIMULI

Feelings of pain not only arise from noxious stimuli applied to the external surface of the body, but also from abnormal conditions within. These stimuli are characterized by being easily, and if intense, supremely in control of reflex responses. Their nature may be thermal, mechanical, chemical, or electrical.

Topography. — On the back of the hand or the forearm outline an area about 2 cm. square. Soften the skin by repeated wetting with warm water, and keep the area moist throughout the experiment. Press the point of a very fine needle firmly upon the skin, but do not pierce the epidermis. Examine systematically the selected area point by point. Be careful to distinguish between sensation just under the needle and sensation from neighboring spots affected by deformation of the skin. Do pain spots and touch spots coincide? (v. Frey, *Ber. d. k. sächs. Gesell. d. Wiss., math.-phys. Kl.*, 1894, p. 185.)

***Latent Period.** — Strike the back of a finger sharply with a small rod or pencil. Is the sensation of pain experienced immediately, or does it arise definitely after the sensation of contact or the hearing of the blow?

Cite, if possible, other examples of a latent period for pain.

***Duration, After-Image.** — In the foregoing experiment did the sensation of pain last longer than the period of application of the stimulus?

In the duration of the sensation is the intensity uniform, or is there an oscillation of the intensity?

Thermal Stimuli. — Fill two beakers with water, one at a temperature of 0°C., the other at 50°C. These temperatures are approximately 25° above and below that of the surface of the fingers. Which temperature is the more painful to the immersed finger?

Which is the more likely to harm the tissues? Remember the effects of heat and cold on frog's muscle.

Chemical Stimuli. — That noxious chemicals, such as strong acids, cause painful sensations has doubtless been experienced.

Referred Sensation. — Stimulate the ulnar nerve at the elbow by moving the ends of the fingers to and fro over the nerve with some pressure. A tingling sensation results likely to be slightly painful. To what region is the sensation referred?

Instead of mechanical stimulation, cold may be used; what is the effect of holding the elbow a moment in a freezing mixture?

Influence of Cocaine. — Press the point of a needle against the tongue, and note the pressure required to cause a distinct sensation of pain. Paint the part with some 5 per cent cocaine. How are the sensations of pressure and pain altered?

THERMAL STIMULI

Only when objects are excessively hot or cold do they give rise to sensations of pain. Within narrower limits the temperature of the object is experienced as warmth or coolness. The end organs influenced by heat or cold are in the skin.

Topography. — Outline an area on the back of the wrist about 2 cm. square. Let a blunt-pointed metal rod stand in cold water until it has become cooled. Dry it and examine point by point the selected area. Mark the spots at which the cool rod causes sensations of cold.

Let the rod stand in hot water until it can be felt as hot when dried and touched to the skin lightly, but not so hot as to cause burning or pain. Explore point by point with very light contact an equal area contiguous to that examined for cold spots. Mark with ink the spots at which the rod causes sensations of warmth. Are the warmth spots as numerous as the cold spots?

Transfer to tissue paper the records made on the skin, and paste the paper in the notebook. (See Blix, *Ztschr. f. Biol.*, 1884, xx, 141; Goldscheider, *Arch. f. Physiol.*, 1885, Suppl., p. 1.)

Intensity. — Warm the metal rod and test on which place a given temperature feels hotter, the palm or the back of the hand; the palm or the mid-line of the forehead; the mid-line or the side of the forehead.

Place the fingers and then the elbow in hot water. Compare the sensations. Which point should be used, for example, in judging the agreeable warmth of water for a bath?

Pour into a beaker water so hot that the touch of the fingers to the outside of the beaker is painful. Sip the water. Compare the skin and the mucous membranes with regard to sensitiveness to heat.

***Adaptation.** — Fill a beaker three fourths full of water not quite hot enough to cause pain. Let the subject hold one index finger in the water for two minutes, and then introduce also the other index finger. Compare the sensations from the same warmth stimulus, applied to normal and to adapted skin.

Let the subject now close his eyes and note carefully the sensations from the two fingers as the beaker is filled with very hot water. The fingers should be lifted as the hot water is added so that fresh areas of skin are not stimulated. Had the end organs for the receiving of warmth stimuli been exhausted in the first finger after two minutes?

To which finger, the adapted or the normal, did the added water feel warmer?

Did the increase of temperature feel the same or different for the two fingers?

Contrast. — Provide three vessels, one of hot, one of cold, and one of lukewarm water. Hold one index finger in the hot, the other in the cold water, for two minutes. Bring them both at once into the lukewarm water. Compare the sensations in the two fingers. Explain.

Repeat the experiment with the middle fingers but immerse them in the hot and cold waters for only one minute. Is the contrast as great as before? Explain.

***Summation.** — Half fill a large beaker with water not quite painfully hot. Insert the hand slowly into the water beginning with the finger-tips. Is the sensation of warmth more intense as more of the hand is stimulated?

***After-Image.** — Hold a cold rod against the forehead for about half a minute. After the rod is removed the temperature of the cooled skin is gradually rising. Is the after-image one of heat or of cold? Recall other examples of the excitation outlasting the stimulus.

MECHANICAL STIMULI

Receptors for mechanical stimuli are found on the surface of the body, where they are sensitive to the contact of objects and give rise to sensations of touch; and they are found within the body where they are sensitive to tensions, as in the contractions of muscles, and sensitive to the movements of fluids, as in the functioning of the semi-circular canals and in hearing. Thus the source of mechanical stimuli may be immediate contact, or may be in the intrinsic activities of body structures, or may be far from the body in objects causing vibrations of the air.

I. Source of Stimuli Immediate

TOUCH

The skin is the sensitive surface not only for noxious stimuli and heat and cold, but also for pressure. Many "tactile sensations" are composites arising from the stimulation of these various receptors, but the most serviceable of them for judging the surface, the consistency, and the form of objects, are the end organs affected by pressure. These end organs, like those for heat and cold, are scattered in the skin, and their distribution can be determined by examination with a blunt pencil point. They are closely related to hair follicles, but occur also in hairless regions.

Relative Sensitiveness of Hairy and Hairless Regions. — Draw the end of a fine thread across the dorsal surface of the last phalanx of the middle finger between the articulation and the nail. Now draw the thread across the dorsal surface of the proximal phalanx of the same finger. Note the difference in the sensation.

Intensity, Threshold of Difference, Weber's Law. — In general the ability to distinguish the differences between different intensities of stimuli, whether heat, cold, pressure, or others, depends not on the absolute difference in any comparison, but on the relative difference. This observation can be proved for any of the senses, but as it was first studied by Weber in relation to the sense of touch, it is of interest to repeat his observations (E. H. Weber, Wagner's "Handwörterbuch d. Physiol.," Braunschweig, 1846, iii (2), 511).

For the following experiment there are required two 50-gm. weights, one 40-gm. weight, two 100-gm. weights, one 80-gm. weight, two 220-gm. weights, one 170-gm. weight, and a small round pasteboard box containing ten 1-gm. weights. The box should have a cover about as heavy as the base. The heavier weights can be made by tying together 10-gm. weights.

Let the subject rest his hand and fingers comfortably, palm up, on the table, and close his eyes. Be careful that the fingers are not supported by muscular contraction during the testing. Place in the cover of the round box the 50-gm. weight, the standard weight for the first test. In the base of the box place the 40-gm. weight. Lay on the two distal phalanges of two adjacent fingers the standard weight; after a moment remove it, and immediately lay on the same surface the weight to be compared. The boxes must be laid on the hand gently and left there the same length of time. The subject must now report, with reference to the tested weight, whether it was "greater" or "equal" or "less," compared with the standard. Always submit the weights in pairs, consisting of the standard and another weight above or below it. The standard should sometimes be first, sometimes second, and each time the subject should be told which is the standard. Occasionally repeat the same weight to make sure that the subject is discriminating.

*Beginning with a weight which is clearly felt as above or below the standard, use in successive tests weights 2 gms. nearer to the standard, until the subject reports, not "greater" or "less," but "equal." Write down in one column the first weight above the standard and in another column the first weight below that are thus reported to be equal to the standard. Now start again at the outer limits and test towards the standard until the report is "equal." Repeat these observations until records of five trials above and five below the standard are secured. This method is known as the method of "least perceptible difference."

Next commence by submitting for comparison the standard weight and a weight greater or less than the standard by 2 gms. Continue thus to increase or decrease the weights until the subject reports, not "equal," but "greater" or "less." When this report is given, write down under the figures obtained by the previous method the weight just perceived as greater and just perceived as less. Repeat the test five times, as before, and set down the results. This method is called the method of "just perceptible difference."

Calculate the average of all the weights just not perceptibly greater and just perceptibly greater than the standard. What is the difference between this average and the standard? Calculate in the same way the average of all the weights just not perceptibly less and just perceptibly less than the standard. What is the difference between this average and the standard? The average of the differences above and below is the threshold of difference of the weight which has been used as a standard.

Determine in the same way the threshold of difference for 100 gms. and for 200 gms. In the latter case the increment or decrement in the tested weight in successive observations may be 5 gms. instead of 2 gms.

What relation is found between a weight and its threshold of difference? Cite other examples of this law in connection with other senses.

The facts of Weber's Law can be simply illustrated by determining, by the method above described, whether the subject can discriminate between 10 and 20 gms., 30 and 40, 40 and 50, 70 and 80, 90 and 100, and 190 and 200 gms. In any doubtful case several trials should be made.

Threshold for Two Points. — Two simultaneous pressures near together on the skin may be felt as one, though they stimulate separate receptors. An area within which this phenomenon occurs is known as a "sensory circle of Weber." The limits of such an area mark the threshold of space for touch.

Let the subject sit with his hand on the table, and with eyes closed. Apply to the back of the hand, gently, equably, and simultaneously, the points of small forceps separated about 5 mm. The subject reports whether he feels one point or two points, or is in doubt. Record the result. Change the distance between the points gradually, in successive tests applied to the same region, until the subject reports a change in sensation. The minimal distance at which the two points can be felt as two points is the threshold (Weber, *Arch. f. Anat., Physiol. u. wiss. Med.*, 1835, p. 152).

Record the results of testing for the threshold on the finger-tips, palm, flexor and extensor surface of the forearm, cheek, and lips.

Draw the forceps along the skin from in front of the ear, across the cheek, and on either side of the lips. Do the points give the same sensation throughout this course? Explain the change of sensation.

***After-Image.** — Stretch a rubber band around the head and allow it to remain for several minutes. What is the sensation when the band is removed?

II. Source of Stimuli Within the Body

(A) MOVEMENT OF MUSCLES AND JOINTS

Histological evidence has proved that muscles are supplied by sensory nerves. This evidence is confirmed physiologically by loss of muscular tone on stimulating the cut nerve of an opposing muscle, by feelings of muscular soreness, and by sensations of weight and resistance when objects are lifted.

***Threshold.**—Let the subject, with eyes closed, lay his relaxed arm comfortably on a board about 45 cm. long. The elbow should be close to one end. Slowly raise or lower the other end of the board, and by means of a millimetre scale roughly determine the amount of the movement before the subject feels the motion. The subject should not mistake sensations of changed pressure or jar for those of motion. Record the amount of movement needed for a just observable sensation, 15 times when the movement is slow, 15 times when it is moderately rapid but not jerky. What is the average of the two series of records? Is there a difference between the threshold for quick and that for slow movements? How do the results compare with other observations on sudden and gradual change of conditions?

Contrast.—Let the forearm and hand rest on the table. Bring the fingers together and turn the hand upright on the ulnar side. Close the eyes and abduct the first finger. The other three fingers will seem to move. In what direction?

Judgment of Weight.—Let a heavy book project over the edge of a table. Lift it twice, first as the hand is slowly moved upward, then as the hand is quickly raised. Does the weight appear lighter or heavier when rapidly raised?

Weber's Law.—State a method of testing Weber's law for muscular movements.

(B) THE SEMICIRCULAR CANALS IN EQUILIBRATION

Although the changes in the semicircular canals do not give rise to sensations which are referred to these organs, they are, nevertheless, intimately connected with important reflexes for the maintenance of body posture, and their functions in this respect can be demonstrated.

Compensating Movements.—Place a frog in a small pan, and cover him with a large beaker or battery jar. Gently rotate the pan. Note the compensating movements, especially of the frog's head. Turn the pan slightly about the transverse, and about the longitudinal axes of the frog's body. What are the effects?

Relation of the Internal Ear to Equilibration.—Etherize the frog. Widely open the mouth. Incise the mucous membrane of the roof of the mouth on the median side of the Eustachian tube. Remove a bit of the cartilage, thus exposing the white otolithic mass. Destroy this mass.

In a second etherized frog destroy both otolithic masses.

After an interval observe the positions of their heads and limbs. Do the animals possess their normal powers of locomotion? How do their swimming movements compare with those of a normal frog? Rotate the animals and look for compensating movements.

Compensating Movements in Man.—Vertigo from whatever cause is a disturbance of the vestibular apparatus either in the labyrinth, or within the brain along the intracranial vestibular pathways. There is a definite normal response to stimulation of the semi-circular canals manifested by a rhythmic jerking of the eyes known as nystagmus, and a subjective sensation of turning called vertigo. Because of this vertigo a subject falls in a definite direction, and when, blindfolded, he attempts to touch an object previously touched, he is unable to find it, but "past points" in a regular manner depending on the direction of the vertigo. In these tests the observer should note the

direction of nystagmus and other changes produced in muscle tonus as evidenced by the pointing error, position of body and the direction of falling. The subject should note the direction of apparent falling, apparent movement of surroundings, and any feeling of nausea. One man should be the subject for the first four parts of the experiment; the observer should then become the subject. A previous demonstration of the method and the results, with explanatory comments, will make the exercise more instructive.

1. On a revolving stool, or Barany chair, rotate the subject 10 times in 20 seconds with his eyes closed. Allow the rotation to stop slowly as the result of friction. Let the subject state the time of stopping and any new sensation of movement that may occur. Compare with the actual facts. Explain.

2. With his head bent forward at an angle of 30° and with his eyes closed, turn the subject to the right 10 times in 20 seconds. Stop suddenly and note the time. Have the subject open his eyes and look straight ahead at some distant point. Note the direction of the slow motion of the nystagmus and its duration in seconds. Repeat in the reverse direction.

3. The subject closes his eyes, touches the observer's finger held in front of him, raises his arm to the perpendicular position, lowers the arm, and attempts to find the observer's finger again. Try with both right and left arm. After rotating 10 times in 10 seconds let him repeat and continue the attempt until the response returns to normal. Correct the pointing after each error. Determine the extent, direction, and duration in seconds of the pointing error.

Repeat again, and correct the error by having the observer move his finger so that it touches that of the subject. Why does this show that the pointing error is a conscious response to the vertigo?

4. Rotate the subject as before. Stop suddenly, allow the subject to stand with feet close together, and note the direction of his tendency to fall. It is the observer's duty to prevent the subject from actually falling.

5. Repeat 2, 3, and 4 with the subject's head bent forward at an angle of 120° and returning to the vertical position at the end of rotation. This tests the vertical semi-circular canals. In which direction should the "past pointing" error be found? How do you test for it? Why?

6. Repeat 5 with the subject's head on his right shoulder. What canals are involved?

Influence of Vision on the Maintenance of Equilibrium. — Try to stand on one foot for a minute with the eyes closed. Repeat the trial with the eyes open. Record the experiences. The method of this experiment is used in principle in the diagnosis of conditions in which the muscle sense is impaired.

III. Source of Stimuli Distant

HEARING

Hearing is the first of the senses in the present consideration which is affected by stimuli originating at a distance from the body. It is next to vision for its importance in expanding the world of stimulations which may influence the bodily activities.

***The Membrana Tympani in Man.** — Fasten the reflector to the forehead and direct the light into the external auditory meatus of the subject. The source of light should be placed about 60 cm. from the reflector, and the reflector about 17 cm. from the ear. Pull the external ear backward and upward, and insert the funnel-shaped tube, with care not to injure the skin of the meatus or the membrana tympani. Concentrate the light on the membrana

by moving the reflector back or forth, and so adjust the tube by tilting it that the whole membrana can be seen.

Near the anterior border of the membrana note the short process of the malleus and its handle extending downward and backward from the short process. Locate the umbo, the most retracted area of the membrane, and locate also the flaccid membrane (Schrapnell's), and the light reflex.

Connection between the Naso-pharynx and the Middle Ear.—Hold the nostrils closed and gently increase the air pressure in the naso-pharynx until the membranæ tympanorum are felt moving outward. Open the nostrils. Does the pressure in the middle ears fall? Swallow. What is the result?

When are the Eustachian tubes open? State one of the functions of occasional deglutition.

Threshold of Hearing.—In a quiet room determine the greatest distance at which the subject, who sits with eyes closed and the right ear plugged with cotton, can hear the ticking of a watch held opposite the left ear. Change the cotton to the left ear and repeat the experiment with the right. The subject should note the varying distinctness of sound when just at the threshold of audibility.

Bone Transmission.—Hold a ticking watch between the teeth. Close both ears with finger-tips. Is the ticking of the watch louder or not? Unstop one ear. In which ear now is the sound louder?

Set the tuning fork vibrating, and hold the stem of it between the teeth. As soon as it has ceased sounding close one or both ears. Is the sound again heard?

Bone transmission of sound is used for diagnostic purposes. What would be its value?

Space Perception.—Let a student click together two coins in various positions with reference to the ears of another student, who acts as subject and keeps his eyes closed. The subject should point in the direction to which he refers the sound. Is the judgment more accurate at the sides of the head or in the median plane?

In the median plane is the reference more accurate in front of the head or above it?

With a finger-tip close the meatus on one side, and observe whether the power of localizing sound is diminished.

***Fatigue.**—Place in the ears the ends of a rubber tube about 1 cm. in diameter and 40 cm. long. Opposite an opening midway in the tube hold a vibrating fork. Pinch the tube near one ear; the sound will now be heard distinctly by the other ear. When the sound has become almost imperceptible reopen the pinched tube. In which ear now is the sound more distinctly heard?

CHEMICAL STIMULI

Chemical stimuli cause two different classes of sensations, according as they affect the receptors of the mouth or the nose. The receptors of the mouth are stimulated by certain non-volatile substances when in solution, the receptors of the nose are stimulated by volatile substances. Since the source of the volatile substances may be more or less remote from the body, the olfactory area of the nose may be regarded, like the ear and the eye, as a distance-receptor.

I. Source of Stimuli Immediate

TASTE

Only certain classes of substances are capable of stimulating the sense organs of taste. These classes and the areas in which they are most effective are studied in the following experiment.

Taste Qualities. Topography. — With a camel's-hair brush apply to different parts of the tongue samples of the following solutions: a solution of quinine sulphate (bitter), a 5 per cent solution of cane sugar (sweet), a 10 per cent solution of NaCl (saline), and a 1 per cent solution of acetic acid (sour). The camel's-hair brush should be washed carefully after each test. Where is each substance tasted most acutely?

The Sapid Substance must be Dissolved. — Wipe the tongue dry and lay on the tip a crystal of sugar. If it is not tasted, wet it. What results? State one of the functions of saliva.

***Threshold.** — Pour into a small beaker about 5 c. c. of a 1:1000 solution of cane sugar. Does the solution taste sweet? Rinse the mouth with fresh water, and repeat the experiment with solutions of the following strengths: — 1:800, 1:600, 1:400, and 1:200. What solution produces a taste which is just perceptibly sweet?

***Influence of a Chemical Agent.** — Apply a 5 per cent decoction of *gymnema sylvestre* to a limited area of the tongue. After 20 or 30 seconds rinse the mouth thoroughly. Now test the taste with the solutions used in Experiment 1. What solutions still produce sensations of taste?

II. Source of Stimuli Distant

SMELL

Taste and smell are frequently confused; especially do substances smelled have their qualities attributed to taste. The confusion can be avoided experimentally by preventing the passage of air through the nose as substances are being tasted in the mouth.

Relations of Taste and Smell. — Place small pieces of apple and raw potato on the tongue of the subject, who during the experiment shuts his eyes and holds his nostrils closed. Is it possible for the subject to distinguish between the two by taste alone? Note the correspondence of this observation with the experience that food loses flavor when the nose is closed by inflammation.

Topography of the Olfactory Area. — Make a sharp-pointed paper cone with the opening at the small end about 3 mm. in diameter. Introduce the small end as far back as possible into the lower region of one nasal cavity until the nostril is plugged. Close the other nostril. Hold the outer large end of the cone over a small piece of cotton wet with ether, or over any other odorous substance, and inspire. Compare the result with the sensation which follows when the inner end of the cone is pointed toward the upper anterior region of the nasal cavity. What indication does this observation give as to the distribution of the olfactory area? (Fick, *Anat. u. Physiol. d. Sinnesorg.*, Lahr, 1864, p. 100.)

***Exhaustion.** — With one nostril stopped, smell tincture of iodine through the other. Hold the bottle near the nose, inhale evenly and somewhat rapidly, and exhale through the mouth.

Does the iodine continue to be smelled? If not, what time has been required to produce exhaustion?

Allow a minute for recuperation and repeat the above test. Repeat until a minute does not suffice for recuperation. What are the successive exhaustion times? Tabulate the results.

PHOTIC STIMULI

The eye, sensitive to rays of light originating in or reflected from objects in the outer world, is the receptor of greatest importance to the organism. The range of objects from which photic stimuli may affect the eye extends all the way from parts of the face in which the eye is situated to the remotest stars, millions of miles away. Not only does this receptor enormously widen the sources of stimulation beyond the limits of the other sense organs, but it furnishes much more accurate data than any of the other sense organs for judging the size and position of objects in space.

The surface specially sensitive to photic stimuli lies in the retina at the back of the eye. The function of the structures in front—the cornea, the lens, the iris—is to bring the light rays, emerging from outer objects, to a focus on the retina. The first problem to be studied is the way in which retinal images are formed.

*Physiological Optics

The essential process of the mechanism by which rays of light are focussed on the retina is refraction. Refraction is a change of direction which a ray of light suffers in passing obliquely through a surface bounding two transparent media of different densities. It is observed that in passing from a less dense into a denser medium the ray is refracted towards a line perpendicular to the surface, and away from the perpendicular line when passing from a denser into a less dense medium. The angle between the incident ray and the perpendicular erected at the point of incidence is the angle of incidence. The angle between the perpendicular and the refracted ray is the angle of refraction. It is observed also that the sines of the angles of incidence and refraction bear a constant ratio to each other for any two given media. And the velocities of light in the two media are related to each other in the same ratio. This constant relation is known as the index of refraction. Since white light is composed of rays of different refrangibility, the index of refraction is expressed with regard to pure spectral rays of intermediate refrangibility, those forming Fraunhofer line D. Usually the velocity of light in air is taken as unity. The index of refraction of water at 15° is 1.332, and of crown glass, used for spectacle lenses, is 1.530. The application of these facts can best be illustrated by constructing in accordance with them the course of a refracted ray.

Students who are familiar with the phenomena of refraction may omit the first three constructions and pass at once to "Refraction in the Eye," p. 62.

Construction of the Path of a Refracted Ray.¹—Draw with ink a line ab 28 cm. long. For convenience let this line coincide with a centimetre division near the middle of a sheet of millimetre paper, 28 cm. long and 6 cm. wide. Select a point c , 12.1 cm. from b . With c as a centre describe toward a , an arc with a radius of 3.9 cm. Designate by h the point of intersection of the arc with ab . Let this arc represent the boundary between air and

¹ For the following constructions there are needed a hard pencil which should be kept sharp, compasses whose pencil point should be kept sharp, and a large sheet of millimetre paper, 44×53 cm. A ruling pen is desirable, but not necessary. Only by accurate drawing will the more complicated constructions give the proper results.

a medium on the concave side of the arc, having the refractive index of water. Draw a line 1.5 cm. above ah and parallel to it, from some point d to a point e in the arc. Let de represent a ray incident to the refracting surface. Draw a line from c to e and extend it to g , an equal distance beyond e . The line ce is a perpendicular to the refracting surface at e . Towards this perpendicular the ray de will be refracted on entering the denser medium. In order to know the direction of the refracted ray it is necessary to know the sine of the angle of incidence.

With the point of incidence as a centre draw arcs with a radius equal to ec ; — one which shall intersect both de and eg , and another which shall pass through c and extend some distance above ab . From the point of intersection, g , draw a line perpendicular to de . If the radius of the arc is regarded as unity, this line is the sine of the angle of incidence.

Note on the millimetre paper the length of this sine of the angle of incidence; if the construction has been properly made, it will be 15 mm. in length. Since the index of refraction of water is 1.332, the sine of the angle of incidence is to the sine of the angle of refraction as 1.332 is to 1.0, or as 4 is to 3. The sine of the angle of refraction will therefore be 11.25 mm. Set the points of the compasses 11.25 mm. apart. With the compasses find a point in the arc above c which will form the centre of an arc tangent to ec and having the radius 11.25 mm. This point marks the intersection of the construction arc with the sine of the angle of refraction. Through this point draw a line from e and continue it until it meets ab . If the drawing has been properly made, it should meet ab at f , 11.6 cm. from c . This is the path of the refracted ray.

Since de was drawn parallel to ab , and the refracting surface is part of a circle, the point f , at which the parallel rays meet, is the posterior principal focus of the curved surface. The point c , to which any ray may pass without change of direction, is called the nodal point.

In constructing the path of a ray refracted in passing from the denser to the less dense medium, the same methods would be used. In this case, however, the ratio of the angle of incidence, now in the denser medium, to the angle of refraction, would be as 1 to 1.332. Draw a line parallel to ab and 1.5 cm. below it, from m in the denser medium to n in the arc separating the two media. Determine by construction the path of this ray on emerging into the air. It should meet the line ab at i , 11.6 cm. from h . The point i is therefore the anterior principal focus of the surface separating the two media. Ink all but the construction lines.

The principles of refraction illustrated by this construction are applicable in all cases in which the index of refraction is known. Thus the course of rays through a lens can be constructed. For an understanding of conditions in the eye and for a knowledge of how a lens affects rays passing through it, making such a construction is desirable. A number of important facts which greatly simplify the relation between an object and its image formed by the lens will be disclosed.

Construction of the Path of a Ray passing through a Lens. — Draw lengthwise through the middle of a sheet of millimetre paper, 19 cm. long and 8 cm. wide, a line op . At the intersection of op with a centimetre division line, 3.5 cm. to the left of the centre of op , set the metal point of the compasses and draw towards p an arc with a radius of 5 cm. The arc should extend 4 cm. on either side of op . Let this arc represent the posterior surface of the lens.¹ From a point 2 cm. to the right of the intersection of the arc with op describe towards o a similar arc. Let this represent the anterior surface of the lens. The line op is now the principal axis of the lens, and on this axis the lens has a thickness of 3 cm.

Draw a line ab , 2 cm. above op , parallel to op , and extending from b on the surface of the lens to a , 7 cm. distant. Let ab represent any incident ray parallel to the principal axis. Draw from the centre of curvature of the anterior surface of the lens a perpendicular to that surface at b . Extend this line several centimetres beyond b . Using the methods already employed for constructing the path of a refracted ray, determine the course which ab will take on entering the lens. Assume that the lens is made of crown glass, with the index of refraction 1.53.

Continue the ray ab as a refracted ray to c on the posterior surface of the lens. Through c draw a perpendicular to this surface from its centre of curvature. Determine the refraction of bc in passing from the lens into air again. It should be refracted to a point f_2 on the

¹ For convenience the lens here constructed is referred to as having anterior points and surfaces towards the left, and posterior points and surfaces towards the right.

principal axis op , 29 mm. from the posterior surface of the lens. Since parallel rays are brought to a focus at the principal focus, and since ab was drawn parallel to op , f_2 is the posterior principal focus. Continue cf_2 until it extends to a point 2 cm. below op . Mark this point d .

From d draw a line parallel to op towards the surface of the lens, which it intersects at e . Construct the path of the ray de after refraction by the lens. The refracted ray comes to the anterior surface of the lens at g . Construct the path of the ray eg after it is refracted by the front surface of the lens. It should meet the principal axis at a point 29 mm. from the anterior surface of the lens. This point is f_1 , the anterior principal focus. Continue gf_1 ; it meets ab at a . Ink the lines representing the course of rays. This construction will reveal many important facts regarding lenses.

Since rays diverging from a are brought to a point at d and vice versa, either a or d might be an object point or an image point, and they are therefore conjugate foci.

Continue the ray ab until it meets in the lens the continuation of the ray cd . They meet at m . Draw a line perpendicular to op through m . This line intersects op at h_2 . The line represents the posterior "principal surface" of the lens, and h_2 is known as the posterior principal point. Similarly continue the ray de until it meets the continuation of the ray ag at n . Draw through n a line perpendicular to op , and intersecting op at h_1 . This is the anterior "principal surface" of the lens. The principal points, h_1 and h_2 , will be about 1 cm. apart and will separate into three approximately equal divisions that part of the principal axis which lies within the lens. The principal surfaces are so related to each other that any ray directed towards a point in one of them seems, after being refracted, to come from a corresponding point in the other. Note that this is true of the just constructed representative rays passing through the lens. In thin lenses the two principal surfaces are so close together that they may be regarded as a single imaginary surface in which the change of direction of a refracted ray takes place. Under these circumstances, for example, a ray, parallel to the principal axis, may be drawn to the principal surface and thence through the principal focus without use of construction lines.

In the lens under consideration the points h_1 and h_2 are important, because they mark the position of the "nodal points" as well as the principal points. Draw perpendiculars through op at f_1 and f_2 . These lines represent the principal focal planes. Mark the point of intersection of the anterior focal plane with ab , by x ; the intersection of the posterior plane with de , by y . Draw a line from x towards h_1 to the anterior surface of the lens. Draw a line from y towards h_2 , to the posterior surface of the lens. Continue these two lines, as dotted lines, to h_1 and h_2 respectively. Now $f_1h_1 = h_2f_2$, and $xf_1 = yf_2$. These right-angled triangles are therefore equal, and since the sides of their right angles are parallel, their hypotenuses are also parallel. The rays xh_1 and h_2y are representative. The nodal points are so related that when any incident ray is directed towards the first nodal point the emergent ray appears to come from the second nodal point and is parallel to the incident ray in direction. As already noted, in this lens, bounded by media of the same refractive index, the nodal points coincide with the principal points. It will be seen later that when a ray enters a refracting system from which it does not emerge, the nodal point does not coincide with the principal point.

Connect by a line the points of intersection of xh_1 and yh_2 with the surfaces of the lens. The line passes between h_1 and h_2 . Where it crosses op it marks the optical centre of the lens. In thin lenses the two nodal points are so close together that they may be regarded

as coinciding with the optical centre. Under these circumstances it is justifiable to represent a ray as passing through the optical centre without change of direction.

Since the posterior focal plane is by construction parallel with the posterior principal surface, and since by construction, $mh_2 = f_2y$, the lines mf_2 and h_2y are parallel. These lines represent the course of rays originating at the point x in the anterior focal plane. All rays arising from any point in the principal focal plane are parallel after passing through the lens.

The two principal foci, the two principal points, and the two nodal points are known as the "cardinal points" of a lens. With these points established it is evidently possible to simplify greatly the construction of the size and position of images formed by lenses. It will be well to apply these facts to some simple conditions in lenses before applying them to conditions in the eye.

Construction of an Image formed by a Convex Lens. — Draw a line to represent the principal axis of a lens.

At right angles to the principal axis near its centre draw a line 2 cm. long, which is bisected by the principal axis. Let this line represent the principal planes of a thin lens, whose principal points coincide with the optical centre. Assume that the principal focal distance of the lens is 3 cm. At any distance beyond the principal focus, *e. g.*, at 5 cm. from the lens, draw an arrow 1 cm. long, at right angles to and bisected by the principal axis.

From the ends of the arrow draw to the line representing the principal planes incident rays parallel to the principal axis, and continue them through the principal focus. From the ends of the arrow draw other rays through the optical centre of the lens. The conjugate foci of the upper and lower points on the arrow are respectively the lower and upper points of the image of the arrow. The image is real and inverted.

Show by similar constructions what will be the relative size of object and image, if the object is at twice the focal distance from the lens? If more than twice the focal distance? As the object is moved nearer to the principal focus what change of size and position occurs in the image?

Virtual Image. — Note what happens when the object is placed between the principal focus and the lens, and the rays whose course is known are drawn. The diverging emergent rays will not form a real image. The perceptive eye will, however, trace these diverging rays back to their meeting point on the same side of the lens as the object, in a virtual image larger than the object.

Hold a convex lens within its focal distance from a printed page. Does the appearance of the letters indicate that a real or a virtual image has been formed? Is the image of the letters smaller or larger than the original?

Hold a concave lens over a printed page. Is the image real or virtual, upright or reversed, larger or smaller, compared with the object? Make a construction showing the course of the rays as affected by the concave lens. In this construction knowledge of principal planes and the optical centres may be applied, but rays parallel to the principal axis are refracted, in passing through the lens, as if directed from the principal focus which is on the same side of the lens with the object.

Determination of the Principal Focal Distance of a Convex Lens. — The focal distance of a lens may be determined by construction, as has previously been done, or it may be calculated from observation.

After removing from the optical lantern the draw-tubes holding the projecting lenses, place in the slot in front of the condensing lens the ground glass screen and the diaphragm with L-shaped aperture. Between this illuminated slit and the screen-block set a mounted convex lens so that an enlarged clear image of the L is thrown on the screen. Measure the length of one limb of the L and let it be represented by o ; the length of its image rep-

resented by i ; and the distance of the screen from the lens, d . Let f represent the principal focal distance of the lens. Construct a diagram showing that the rays from the ends of the L-shaped slit, which are refracted through the principal focus, form similar triangles between the lens and the screen-block. Then the following proportion is true:—

$$\begin{aligned} o : i &:: f : d - f; \\ \text{and} \quad if &= od - of, \\ \text{and} \quad if + of &= od; \\ \text{whence } f &= \frac{od}{i + o} = d \frac{o}{i + o} \end{aligned}$$

Thus, by knowing the length of the object, the length of its image, and the distance of the image from the lens, it is possible to calculate the principal focal distance of the lens.

Refraction in the Eye.—Rays of light entering the eye pass through five refracting media, the layer of tears, cornea, aqueous humor, lens, and vitreous humor. It is not necessary, however, to consider each of the surfaces separating these media as a refracting surface, for the indices of refraction of the layer of tears, the cornea, and the aqueous and vitreous humors are practically the same, approximately 1.336. The lens has a higher refracting power near its centre than in its outer layers, but without considerable error the lens may be regarded as a homogeneous structure with a refractive index of 1.437. The eye, therefore, may be regarded as composed of a transparent refractive medium containing a lens of slightly higher refractive power. And rays entering the eye would be refracted by the anterior curved surface of the medium, the cornea, and by the front and back surfaces of the included lens.

These refracting surfaces form practically a “centred system,” *i. e.*, the axes of the refracting surfaces lie in the same straight line. In any centred system, even if it is composed of many refracting surfaces, it is possible to determine the two principal foci, the principal points, and the nodal points of the system, and thus greatly simplify the consideration of the passage of rays through it. In determining these six cardinal points for the eye, it will be possible to apply the knowledge of cardinal points already secured in the study of refraction by a convex lens.

The refractive surfaces of the eye are sometimes regarded as belonging to two systems: system A, the cornea, and system B, the lens. The combination of the two systems, *i. e.*, the eye, is then called system C. For convenience this classification will be used in the following directions.

In the first construction in this study of physiological optics the refracting surface used was a five-fold magnification of the cornea (see p. 58). The principal surface, the nodal point, and the anterior and posterior focal distances of system A have therefore been determined. In correspondence with that construction, the construction showing the course of rays through the eye will also be made five times the natural size. It should be remembered that the measurements here given are averages of numerous observations.

On a sheet of millimetre paper 52 cm. long and 8 cm. wide draw a line xy coinciding with the centimetre division midway in the width of the paper. Let xy be the axis of the centred system. With a point 25.1 cm. from y as a centre describe an arc towards x , with a radius (7.8 mm. \times 5) 3.9 cm. Let the arc, which represents the cornea, extend 2 cm. above and below xy . The centre of curvature of the arc mark Ak , this is the nodal point of sys-

tem A. The point of intersection of the arc with xy is Ah , the principal point of system A. Note that in system A, in which only one refracting surface is presented, and the ray entering the medium does not emerge, there is only one nodal point, which coincides with the optical centre, but does not coincide with the principal point.

Reference to the earlier construction will show that the posterior principal focus of system A was found (23.2 mm. \times 5) 11.6 cm. posterior to Ah . Erect at this point, Af_2 , a plane perpendicular to xy , which will be the posterior focal plane of system A. The anterior principal focus was found (23.2 mm. \times 5) 11.6 cm. in front of Ah . Erect at this point, Af_1 , a plane perpendicular to xy , to represent the anterior focal plane of system A.

The anterior surface of the lens is 3.6 mm. back of the anterior surface of the cornea. The radius of curvature of the anterior surface of the lens is 10 mm. With a point (13.6 mm. \times 5) 6.8 cm. posterior to Ah describe an arc towards Ah , with a radius of 5 cm. This arc represents the anterior surface of the lens.

The thickness of the lens at rest is 3.6 mm. The radius of curvature of the posterior surface of the lens is 6 mm. The centre of curvature of the posterior lens surface is, therefore, (6 - 3.6) 2.4 mm. anterior to the point of intersection of the anterior lens surface with xy . With the point (2.4 mm. \times 5) 1.2 cm. anterior to this point of intersection as a centre describe an arc towards y , having a radius of 3 cm. Let this arc, representing the posterior surface of the lens, intersect the arc representing the anterior surface of the lens. The construction now shows accurately the shape and relations of the lens and cornea of the eye on a five-fold scale.

As previously noted, the lens, with a refractive index of 1.43, is surrounded by a medium with a refractive index of 1.33. The effective refracting power of the lens as it rests between the aqueous and vitreous humors is, therefore, only $\frac{1.43}{1.33} = 1.075$. It can have rela-

tively little effect in changing the direction of the rays passing through it, and its focal distances are accordingly long. The two focal distances are the same (50.61 mm.).

The principal points of the lens coincide with the nodal points, and they can be found by the method used in the construction of the path of a ray refracted by a biconvex lens. The anterior principal point is within the lens (2.12 mm. \times 5) 1.06 cm. from the anterior surface; the posterior principal point is within the lens (1.27 mm. \times 5) 0.63 cm. from the posterior surface. Find these points, Bh_1 and Bh_2 respectively, and draw the principal surfaces perpendicular to xy . From Bh_2 measure (50.61 mm. \times 5) 25.3 cm. towards y , and thus find the posterior principal focus, Bf_2 . Draw a line through Bf_2 perpendicular to xy , to represent the principal focal plane. Mark the anterior principal focus of the lens Bf_1 , 25.3 cm. from Bh_1 . All of the cardinal points of systems A and B are now known.

From a point a in the air, 1.5 cm. above xy , draw a line to b in the cornea, parallel to xy . Let the line ab represent an incident ray. This ray, parallel to the principal axis, will be refracted towards the principal focus, Af_2 . Continue ab towards Af_2 from b to c in the anterior principal surface of the lens, and thence, parallel to the principal axis, to d in the posterior principal surface.

To find the direction which the ray will take from d , extend the line bc 5 or 6 cm. beyond b , and draw to Bh_1 a line parallel to bc . This line, representing a ray directed towards the first nodal point, will be continued as from the second nodal point, Bh_2 , without change of direction. Continue the line as if from Bh_2 until it intersects the posterior principal focal plane of system B, at z . Since parallel rays are brought to a focus in the

principal focal plane, draw the line bcd from d towards z until it meets xy . It should meet xy (22.8 mm. \times 5) 11.4 cm. from Ah , at the posterior principal focus of system C, Cf_2 .

Draw a line parallel to xy from a point e in the posterior focal plane of system C, 1.5 cm. below xy , to g in the anterior principal surface of the lens. The incident ray represented by eg will be refracted towards the anterior principal focus of system B at Bf_1 . Draw from g a line towards Bf_1 to i in the surface of the cornea. This ray, gi , will now be refracted by the cornea.

To find the course of the ray gi from i , extend the line several centimetres beyond g and draw through Ak to a point s in the anterior focal plane of system A, a line parallel to gi . Since parallel rays are focussed in the focal plane, continue egi towards s , until it meets the principal axis xy . It should meet xy (13.7 mm. \times 5) 6.85 cm. in front of the cornea, at the anterior principal focus of system C, Cf_1 . Thus two of the cardinal points of the eye, the principal foci, may be determined. At these points rays parallel in the air, or in the vitreous humor, will be brought to a focus, and rays emerging from any point in either of the principal focal planes will be parallel after the last refraction.

To find the principal surfaces of system C, proceed as follows. Prolong the lines dCf_2 and ab until they meet. The meeting point lies in the posterior principal surface. Draw a line through this point perpendicular to xy . The point of intersection will be the posterior principal point of system C, Ch_2 , (2.11 mm. \times 5) 10.55 mm. behind Ah in the anterior surface of the cornea.

Similarly prolong the line eg until it meets the extension of iCf_1 . Mark the point of intersection t . It lies in the anterior principal surface. To represent this surface draw a line through t perpendicular to xy . The point at which this line crosses xy is Ch_1 , the anterior principal point of system C, which lies (1.75 mm. \times 5) 8.75 mm. behind Ah in the anterior surface of the cornea. The distance between the two surfaces is (0.36 mm. \times 5) 1.8 mm.

The principal surfaces possess the same properties in system C that they possess in any simple convex lens. They are so related that any ray directed towards a point in one surface may be drawn as coming from a similar point in the other surface, and any line parallel to the principal axis and directed to a point in one principal surface may be drawn as coming from a similar point in the other and passing to the principal focus.

Of the six cardinal points of the eye only the nodal points remain to be determined. With system C, as with system A, the ray entering the system does not emerge from it. And in system C the nodal points do not coincide with the principal points, just as in system A the nodal point did not coincide with the principal point. The position of the nodal points of system C can be found by construction.

Let the point e , previously noted, represent any point in the posterior focal plane. The ray et , already constructed parallel to the principal axis, is refracted to meet that axis at Cf_1 . Any other ray emerging from e will be refracted so as to be parallel to tCf_1 . A ray from e parallel to tCf_1 would therefore be continued in the same direction after refraction. From Cf_2 measure towards Cf_1 (15.4 mm. \times 5) 7.7 cm., a distance equal to the anterior focal distance, Cf_1h_1 . Mark the point thus found Ck_2 . Draw a line eCk_2 , and extend it until it intersects the posterior principal surface of system C. Mark the point m . The ray will pass from m to a corresponding point, n , in the anterior principal surface, and thence to o , in the anterior focal plane, in a direction parallel to tCf_1 . But, since by construction the triangles tCf_1h_1 and eCk_2f_2 are equal right-angled triangles, with the right-angled sides

parallel, their hypotenuses must also be parallel. The line eCk_2 is therefore parallel to tCt_1 , and is consequently parallel to no . Continue no from n until it meets xy . Mark the point Ck_1 . Since mn is parallel to xy , and eCk_2 is parallel to no , the distances mn and Ck_1k_2 are equal. The points Ck_1k_2 are the nodal points of system C, for they are so situated that when an incident ray is directed towards one, the corresponding refracted ray seems to come from the other and is parallel to the incident ray. The two nodal points are separated by the same distance as the two principal points, $(0.36 \text{ mm.} \times 5) 1.8 \text{ mm.}$

The "Reduced" or "Schematic" Eye.—The foregoing construction has shown that in the normal eye the two principal surfaces and the two nodal points are only $(1.8 \text{ mm.} \div 5) 0.36 \text{ mm.}$ apart. This distance is so small that it is justifiable to combine the two principal planes in a single principal plane, and the two nodal points in a single nodal point. In the above construction of system C the principal planes were approximately 25 mm. from the nodal points; one-fifth of this is 5 mm., the distance from the combined principal surfaces to the combined nodal points. The combined nodal points are situated $(7.75 \text{ cm.} \div 5) 15.5 \text{ mm.}$ in front of the posterior principal focus. Thus, without important alteration of the facts of refraction in the eye, its refracting mechanism can be simplified to a single nodal point 15 mm. in front of the posterior principal focus, a principal surface 5 mm. in front of the nodal point, and an anterior principal focus 15 mm. in front of the principal point. This is the reduced or schematic eye. Rays pass through the nodal point without change of direction; rays parallel to the principal axis are refracted in the principal surface towards the principal focus. All rays from any point in a principal focal plane are refracted in the principal surface so that they are parallel. These principles are the same in the simplified system of the eye as they are in the simplest dioptric system. By means of them it is possible to understand with clearness and accuracy the eye and its manner of affecting the rays entering and leaving it (Listing, Wagner's *Handwörterbuch d. Physiol.*, 1853, iv, p. 493).

The Size of the Retinal Image.—In crossing at the nodal point the rays from different parts of an object make similar triangles. Calculate the size of the retinal image formed by an object 1.5 metres high which is 450 metres from the eye.

Observation of the Retinal Image.—The foregoing discussion and constructions have shown the way in which rays entering the eye are refracted so as to produce an image on the retina. The image thus formed can be viewed objectively.

Remove the eyes from a white rabbit and fix each in a ring of modelling wax. In a darkened room hold about 50 cm. before one of the eyes a lighted match or gas flame. Look at the back of the eye. Is the image upright or inverted? Blow the flame and note the change in the image.

The same observation may be made on a light-haired, blue-eyed person. Let him look as far as possible towards the left. Hold a lighted match or gas flame on the extreme temporal side of the left eye. Note the image shining through the sclera on the nasal side of the eye. Is it upright or inverted? (Helmholtz, *Physiol. Optik*, Leipzig, 1896, p. 86.)

Retinal Images are referred to an External Source through the Nodal Point.—That retinal images are referred outward through the nodal point might be inferred from the fact that rays traversing this point are not changed in direction.

Close one eye and turn it as far as possible towards the nose. Near the orbit on the temporal side press gently against the eyeball with the tip of the little finger, first through the upper, then through the lower lid. The retina is mechanically stimulated beneath the pressure. Where does the origin of the image (the phosphene) appear to be?

Accommodation

The constructions and discussions of ocular refraction have hitherto been concerned with parallel rays, which on entering the resting eye are brought to a focus on the retina. If rays entering the resting eye are not parallel, but divergent, they no longer focus on the retina.

Draw the principal axis, the principal surface, the posterior focal plane, and the nodal point, which represent the reduced eye. From a point *o* in the air, about 10 cm. in front of the eye, draw a ray through the nodal point. Draw another ray parallel with the principal axis, from *o* to the principal surface, and thence through the principal focus. Prolong the two rays until they meet. Where do diverging rays focus when they come to the resting eye? Since it is possible to see clearly near objects from which divergent rays come to the eye, a mechanism must be present which can increase the ocular refraction and shift the position of the focal plane of the eye. This power of altering the focus is called accommodation.

The Range of Accommodation. — That the power of bringing diverging rays to a focus is limited is shown by the following experiment. Hold a needle at arm's length from the eye and gradually bring it nearer. It is clearly seen only to a certain point; then it begins to become blurred, even though a strong attempt is made to maintain distinct vision. The point at which the maximum degree of accommodation is attained is called the near point. Measure the distance of the near point from the eye.

The far point is normally at an infinite distance, but the myopic eye (p. 69) has a measurable far point beyond which objects distinctly visible to the normal eye appear blurred.

The distance between the near and far points measures the range of accommodation.

***The Accommodation Line.** — In a strip of wood set pins upright at different distances from the eye, 25, 50, and 100 cm. At each distance note with one eye how far a second pin can be set beyond the first in the visual line without loss of distinct vision of either pin. The greatest distance between two objects on the visual line within which the two can be clearly seen at the same time is called the accommodation line. Does this line lengthen as the two objects are set farther from the eye? (Czermak, *Verh. d. phys.-med. Gesell., Wurzburg*, 1850, i, 185.)

The Nature of Images not in Focus, Scheiner's Experiment. — If the eye is accommodated for an object at any particular distance, it is clear that objects nearer to or farther from the eye than that object will not be in focus unless they lie in the same accommodation line with it. The way in which the light comes to the retina from objects not in focus is seen in Scheiner's experiment (Scheiner, *Oculus, sive fundamentum opticum*, Innsbruck, 1619, Lib. iii, p. 163).

***Observations on Rays passing through a Lens.** — Set in front of the condenser of the lantern the ground-glass screen and the diaphragm with a vertical slit. Through the 10 D

lens throw an image of the slit on the black wooden block. Now place against the lens a sheet of cardboard perforated with two holes, 3 mm. in diameter, horizontally related, and less than the diameter of the lens apart. What change is observed in the appearance of the image? Cover one of the holes and record the result.

Move the object farther from the lens, and note the result. Cover the left hole. Which image is affected, the right or the left?

Move the object nearer to the lens than the original position. Again, how is the image affected, and what change occurs when the left hole is covered?

Make diagrams showing the course of the rays in each of these conditions, and explain in each instance the result of obstructing the passage of light through one of the holes.

Observations on Rays entering the Eye.—In a thin piece of cardboard prick two small holes not more than 2 mm. apart. Bring the holes horizontally in front of the pupil and look along a strip of wood at two pins standing upright, one 30, the other 60 cm. from the eye. If the eye is accommodated for the farther pin, how does the nearer pin appear? While the eye is still focussed on the farther pin, cover one of the holes. Record which hole was covered and the resulting change in what is seen.

Repeat the experiment with the eye focussed on the nearer pin. What change now occurs when the same hole is covered as before?

Draw diagrams explaining the observed phenomena. Remember that in referring retinal images to the outer world the reference is always from points on the retina outward through the nodal point.

Circles of Dispersion.—It is clear that if the cardboard in either of the two foregoing experiments were removed, the light from the object not in focus would not be limited to the small holes for entrance into the eye, but would fill the entire lens or pupil. And rays from a single point that are not united in a focus must illuminate a circle, not a point. These circles are called circles of dispersion. Such dispersion circles were formed on the retina in uncompensated myopia and hypermetropia (p. 69). Recall the common experience of looking through the netting in a window. To what extent do the blurred images of objects not in focus ordinarily receive attention?

***Action of the Iris in Accommodation.**—Already in observations on reflexes in man the fact has been noted that when the gaze is transferred from a distant to a near object there is normally a narrowing of the pupil—the iris contracts. The observation on spherical aberration proved that as the rays entering a lens become more divergent the aberration is increased, and also that the aberration is especially due to rays refracted at some distance from the principal axis. What is the advantage, therefore, of the contraction of the iris in accommodation?

The changes of the pupil in accommodation each person can observe in his own eyes. Make a pin hole in a card, and, holding the card close to the near point of the right eye, look through the hole towards a light. Hold the hand over the left eye, and accommodate for the distance of the card. Note the size of the hole. Now look far away as if gazing through the card. What apparent change occurs in the size of the hole? Recall Scheiner's experiment.

Hold the pin hole at the anterior principal focus of the right eye. While accommodating for a distance cover the left eye. When light is excluded from the left eye, what change occurs in the appearance of the pin hole? Uncover the left eye and note the change. Explain these alterations in the apparent size of the pin hole.

***Changes in the Lens in Accommodation.** — The essential change in the eye, the change that brings the focus of divergent rays forward to the retina, occurs in the lens. This change has been studied by means of the reflections from the different refracting surfaces of the eye, for not all of the light coming to the eye reaches the retina.

In a dark room hold a watch glass (convexity forward) a few centimetres in front of the 10 D lens. The axis of the lens and watch glass should lie midway between a very low gas flame and the observer's eye. Note the images reflected from the surfaces of the watch glass, the anterior surface of the lens, and the posterior surface of the lens. In what order do they appear in a horizontal series? What is the source of each image? Which is inverted?

Repeat this observation on the eye. Hold the low gas flame (about the size of a candle flame) so that it and the observer's eye are symmetrically placed with reference to the optical axis of the observed eye. Let the subject fix his gaze at some distinct distant object. By careful adjustment three images can be seen: a bright one on the flame side; another bright one, small and inverted, on the side of the observer; and a faint, enlarged, upright image between these two. Let the subject hold a pin close to his near point and between the eye and the object he has been gazing at. As he accommodates for this near object try to note what change occurs in the images. The faint middle image should move towards the flame and become smaller. Using the observations on the watch glass and the lens as an analogy to the eye, explain the source of the images observed in the eye. How could the changes in the middle image be explained? (Purkinje, *De examine physiologico organi visus*, Breslau, 1823.)

Let the subject cover one eye and with the other look at a distant point. Let the observer view the subject's eye in profile but somewhat obliquely so that he can see just the farther side of the iris. Now the subject should hold a pin between the eye and the point he has been gazing at, and should adjust his vision to the near object. At the moment the accommodation is effected, more of the pupil shows and the iris seems to become narrower. Is any change observed in the distance between the front of the cornea and the profile of the pupil? Do the observations just made indicate an alteration in the form of the lens, effective for accommodation? Explain.

Defects of the Eye

Thus far in the study of the receptor for photic stimuli, it has been assumed that the surfaces of refraction are truly spherical, and that parallel rays are always brought to a focus on the retina. These conditions are not perfectly realized in the eye, and frequently are so far from realization as to produce noteworthy defects of vision. Some of these defects the eye shares with other dioptric systems, others are peculiar to the eye.

***Spherical Aberration.** — A spherical lens does not refract all rays coming from a point exactly to a point. Rays refracted by the outer portions of such a lens are brought to a focus nearer the lens than those refracted by portions close to the principal axis.

Cover with a glass slide the round opening in the end of the optical box. Set in the box close to the window a cylindrical bottle full of clear water. Light the incense in the cork, cover the box, and let it fill with smoke.

In front of the condenser of the optical lantern set the diaphragm with a 2 mm. opening. Remove the projecting lenses. Place the lantern about a metre from the optical box so that the rays pass through the bottle, which acts as a refracting cylinder. Look directly down upon the refracted rays. Note that the two boundaries of the lighted region are not plane surfaces extending from the sides of the bottle to the focal line, but are curved surfaces. These curved or caustic surfaces are due to the outer rays being more refracted than the inner, and therefore intersecting the inner rays.

Bring the lantern nearer the bottle. Is the curvature of the caustic surfaces increased or diminished? Interrupt the outer rays coming to the bottle. Does the focus become more distinct? Note the greater importance of intercepting the outer rays, as the incident rays become more divergent.

The rays which focus in front of the principal surface continue their course and tend to blur the outlines of the image formed by the more central rays. Set the screen at the linear focus of the bottle. The region either side of the focus is dimly illuminated.

Chromatic Aberration.—That a prism not only refracts light, but refracts differently the waves of different wave length is well known. The red rays with a greater wave length are less refracted than the violet waves with a less wave length. A lens may be regarded as an infinite series of prisms; and therefore the same analysis of white light would be produced by a lens as would be produced by a prism. When a source of white light sends rays to a convex lens the violet rays, most refracted, are focussed nearer the lens than the red rays, and the foci of the other spectral rays lie in a band of colors between these extremes. The existence of chromatic aberration in a lens and in the eye can be readily demonstrated.

Set in front of the condenser of the lantern the ground-glass screen and the diaphragm with a 2 mm. aperture. Place the block holding the convex lens approximately 15 cm. from the diaphragm. The image of the illuminated opening, when thrown on a white screen, will be, in a position nearer the lens than the focus, a disc with violet centre and red border. Move the screen beyond the focus. The colors in the disc now change position; there is a red centre and a violet border. Draw a diagram explaining these observations.

Cover with a card one half the pupil and look at a distant window or at an electric light filament. These objects now seem to have colored fringes. What are the colors and what are their relations to each other? (Fick, *Anat. u. Physiol. d. Sinnesorg.*, 1864, p. 240.)

Chromatic aberration is greatly reduced by cutting off the outer rays by a diaphragm. Cover the lens in the above experiment by a card with a 1 cm. opening. Note that the image becomes more distinct and to a large extent loses its colors.

Emmetropia, Myopia, Hypermetropia, and the Numbering of Lenses.—The normal eye in which parallel rays are focussed on the retina is called emmetropic. In abnormal eyes the principal focus may be in front of the retina or behind it; when in front, the vision is said to be myopic; when behind the retina, hypermetropic.

The degree of myopia or hypermetropia is measured by the refractive power of the lens required to bring the principal focus exactly on the retina. The refractive power of lenses

is expressed with reference to a lens with a focal length of one metre. This unit is called a diopter, usually written D. Thus a lens of two diopters has twice the strength of the unit, *i. e.*, its focal length is half a metre; and a lens with a focal distance of two metres is 0.5 D. Convex lenses are marked +, concave lenses -. What is the focal distance of a lens of 5 D? The focal distance of the convex lens in the block is 10 cm. Express its power in diopters.

Place the block holding this lens inside the optical box close to the window. Set before the condensing lens the diaphragm with 2 mm. aperture and arrange the draw-tubes of the optical lantern so that they project a beam of light of approximately uniform diameter. Let this light pass through the lens into the box. Move the screen away from the lens about 2.5 cm. beyond the principal focus. What defect of the eye is now illustrated? Hold in front of the window of the box the weak concave lens marked -2 D. What is the change in the image? Why was a concave lens used? What is the degree of the defect in this instance?

Place the screen 2.5 cm. nearer to the lens than the principal focus. What ocular defect is now illustrated? Use as a corrective lens that marked +2 D. Does the image become clear? Explain the use of the convex glass. What is the degree of the defect in this instance?

Astigmatism.—Hypermetropia is almost always due to the eyeball being too short, and myopia to its being too long. In neither of these conditions is there necessarily any deviation of the refracting surfaces from the normal shape. The normal shape of the cornea is not in fact spherical but ellipsoidal, with the focus of the vertical meridian slightly shorter than the horizontal. This fact can be proved by drawing on a card two lines at right angles, holding the card close to the eye and slowly moving it away. The horizontal line will be clearly seen at a shorter distance than the vertical. Some astigmatism therefore exists in the normal eye. It is an exaggeration of this condition which is the usual form of "regular astigmatism." The curvature of the cornea differs in the two meridians which are at right angles to each other. Thus the vertical meridian may be emmetropic; the horizontal, hypermetropic.

Set at one end of the table the optical box containing the screen, and at the other end the optical lantern without diaphragms or draw-tubes. Place in the box near the window the convex lens (10 D) in the axis of the beam. The image on the screen will be circular. Set outside the box close to the convex lens, the block holding the cylindrical lens, with the curvature vertical. What change occurs in the image? Move the screen gradually nearer the lenses and note the changes in the shape of the image. With the convex lens still near the window, fill the optical box with smoke. Start with the curvature of the cylindrical lens in the vertical meridian, and slowly rotate the block. Note carefully the change in the shape of the beam of light as the lens is turned. Make a diagram showing the course which the rays take when one meridian refracts more than that at right angles to it, and showing how two linear foci can be formed from a point of light. What must be the effect of this condition on the clearness of vision?

Set before a window a square of cardboard in which there is a rayed figure made by holes of uniform size. Are some of the rays seen clearly and some blurred? If all the rays are seen clearly, note the effect of looking at the clock through a cylindrical lens. Record the appearances. Explain.

***Ophthalmoscopy**

The reason that we see any object is because light rays come from it to our eyes. As has been seen, light entering the eye is in part reflected from the various surfaces, and in part refracted. Of the light which reaches the retina, part is absorbed by the choroid coat and part is reflected. Rays which are brought to any point on the retina must emerge from the eye, however, along exactly the same course by which they entered, and will be refracted back to their source. The pupil ordinarily appears dark because no light is coming through it to the observer's eye. And no light is reflected to the observer's eye because the pupil of the observer's eye is dark and does not illuminate the observed eye. In order to render the interior of the observed eye visible, it must be illuminated as if by light coming from the observer's eye. This effect is produced by means of the ophthalmoscope (Helmholtz, *Beschreibung eines Augenspiegels z. Unters. d. Netzhaut im lebenden Auge*, Berlin, 1851).

The Ophthalmoscope. — The ophthalmoscope, invented by Helmholtz in 1851, consists essentially of a mirror arranged to reflect light into the observed eye while the observer, looking through a central opening in the mirror, views the illuminated retina and its vessels. In most ophthalmoscopes there is an arrangement for bringing over the central opening lenses of different refractive power by which the rays passing between the two retinæ may be refracted. In the Loring ophthalmoscope these lenses are borne in two revolving discs, which are so disposed that the lenses may be used singly or in combination. Thus it is possible to secure variations of refractive power from $+0.5D$ or $-0.5D$ to $-24D$ and $+23D$.

The Influence of the Size of the Pupil in the Observed Eye. — Draw a reduced eye, and just back of the principal surface represent the iris with an opening 4 mm. in diameter. Assume that the point on the principal axis, 10 cm. in front of the principal surface, is a source of light. Show by construction how large will be the diameter of the illuminated area on the retina.

Repeat the construction but make the diameter of the pupil 8 mm. What is the effect of the change on the size of the illuminated area? If the nodal point of the observer's eye were placed at the point where the source of light was situated in this experiment, what would have been the effect of enlarging the pupil on the size of the visible area in the retina?

The Influence of Proximity of the Observed and the Observer's Eyes. — On the same axis draw two reduced eyes facing each other at a distance of 10 cm. Let the pupil of each eye be 4 mm. in diameter. From the borders of the pupil of the observer's eye draw lines through the nodal point of the observed eye. What is the diameter of the visible area in the retina of the observed eye?

Repeat the construction but let the distance between the eyes be only 5 cm. Is the size of the visible area thereby changed?

The Direct Method of using the Ophthalmoscope. — In the direct method of using the ophthalmoscope rays pass from one eye to the other without the interposition of any refracting medium. The two foregoing constructions have shown the importance of having the pupil of the observed eye large and close to the observer's eye. The pupil may be dilated without affecting accommodation by dropping on the eye a 3 per cent solution of cocaine. A drop or two of the solution is sufficient. Keep the lids closed and shaded from the light for about fifteen minutes until the cocaine has had its effect.

While the cocaine is becoming effective in the subject's eye examine with the ophthalmoscope the artificial eye. Draw out the inner tube of the artificial eye to the line marked zero, and set it on a standard. At the same level, but to the right of the eye and slightly behind it, place a gas flame. As the observer looks into the artificial eye he should face the flat surface of the flame. Hold the ophthalmoscope in the right hand close to the eye and look through the hole in the mirror. By means of the mirror reflect the light into the artificial eye. After finding the red reflection of the fundus, gradually approach the artificial eye until the ophthalmoscope is in the anterior principal focus (5 cm. in front of this eye). As the artificial eye with the tubes at zero is arranged to represent the resting eye, the observer should relax his accommodation, and gaze as if at some distant object lying far beyond the eye before him. Then the parallel rays emerging from the model are focussed on the observer's retina without effort. As soon as the branching blood vessels are seen, trace them to their source in the optic disc, and outwards as far as possible in their distribution.

Repeat this observation on the eye of the subject whose pupil is now enlarged by cocaine. The subject should look straight forward and should not accommodate. Use the left eye for observing the subject's left eye; and similarly the right eye for the subject's right eye. Bring the light close to the mirror, and the mirror as close as possible to the eye. If the two eyes are now fully relaxed a clear view of the details of the retina may be obtained without any effort. Find the optic disc and trace the larger blood vessels passing out from it.

If the accommodation of the two eyes is quite relaxed and yet the image is not clear, the condition must be one of defective refraction. A skilful observer with a resting emmetropic eye can judge in such cases the amount of error of refraction by means of the lens required to make clear the image of the subject's retina. If there is time, judging the experimental hypermetropia or myopia of the artificial eye in this manner may be tried.

Draw a diagram showing the course of rays arising from an object on the surface of the retina and passing to the observer's eye. What is the relation of the image to the object in this instance? The mirror throws converging rays into the eye; in a separate diagram show the course of these rays.

The Indirect Method of using the Ophthalmoscope. — In the indirect method of using the ophthalmoscope the light from the subject's eye is intercepted by a strong convex lens (5 cm. focal length) held near the eye; and the image formed by the lens is then observed. A larger part of the retina can be seen at one time by this method but the magnification is less than with the direct method.

Hold the 20 D convex lens 5 cm. in front of the artificial eye, and, with the ophthalmoscope about 25 cm. still farther in front, throw rays from the mirror through the lens upon the retina. Look through the opening in the mirror at the image of the retinal structures formed by the convex lens. Turn a +5 D lens into the opening in the mirror, and again examine the image.

Repeat this observation on the subject's eye. How does the appearance of the retina compare with the appearance when the direct method is used?

Draw a diagram showing the course of the rays in the indirect method of using the ophthalmoscope. Neglect the course of the rays from the mirror to the observed eye.

Some of the Conditions of Vision

The apparatus for producing a clear image on the retina is essential for vision, but that image must affect the sensory endings of the nerves in the retina or no sensation of light results. Many characteristics of retinal stimulation can be shown by experimental means. Only a few of the more important observations can be considered here.

The Blind Spot.—Where the optic nerve enters the eye the rods and cones are absent. The region therefore is insensitive to light.

Fasten a rod 30 cm. above a sheet of white paper lying on the table. Rest the chin on the rod, close the left eye, and look directly down with the right eye at a cross marked on the paper. Let the experimenter cement a small piece of black paper to the end of a straw, and draw the black object along the paper towards the temporal side of the eye which is being tested. Mark on the paper where the black ceases to be visible and where it reappears. Determine its boundaries in several diameters. Great care should be taken to keep the gaze fixed on the cross on the paper. (See E. H. Weber, *Ber. d. k. sächs. Gesell. d. Wiss.*, 1853, pp. 149–153.)

How long is the greatest diameter of the blind spot?

***The Blood Vessels of the Retina.**—Not only the optic nerve, but also the blood vessels of the retina enter the eye at the blind spot. The vessels are distributed on the front surface of the nervous layer, and under certain circumstances the shadows they throw on the sensitive layer may be observed.

Press the tips of the thumbs and index fingers together so as to enclose a small hole about 1 mm. across. With one eye look at a whitened wall or at the blue sky through the small hole, which should be moved close in front of the eye with a circular motion. Soon a punctated field appears, webbed with branching lines—the blood vessels of the retina. Note that the blood vessels do not enter a central region in the field. Move the hole vertically,—what vessels become more distinct? Move the hole horizontally,—what vessels now become more distinct? Draw a diagram to show how these retinal vessels become visible.

The blood vessels may be seen over a larger area of the retina by looking towards a blank wall in a dark room while a candle or the light from the bottom of the optical lantern at one side of the eye is gently moved in a small circle. In a few moments a widely spreading network of vessels is seen projected into space. From the position of the projected figure judge where in the retina the shadows of the blood vessels must fall. (Purkinje, *Beobacht. u. Versuche z. Physiol. d. Sinne*, Berlin, 1823–25; H. Müller, *Verh. d. phys.-med. Gesell.*, Würzburg, 1855, v, 411. Purkinje first observed these phenomena, they were more carefully examined by Müller.)

***The Yellow Spot.**—The foregoing experiment showed that there is a region of the retina close to the line of vision which is lacking in blood vessels. This region is colored by a pigment absorbing blue and green and appearing therefore reddish yellow. This is the “yellow spot.”

Close the eyes for a minute, then with one of them look towards a white cloud through a flat bottle filled with a strong solution of chrome alum. This solution transmits blue, green, and red rays. Explain the appearance observed in the visual field in this experiment. (Maxwell, *Rep. Brit. Assn.*, 1856, ii, 12.)

***Muscae Volitantes.**—Again make the small hole by pressing together the tips of the thumbs and index fingers. Holding it slightly more than a centimetre in front of the eye, look at an evenly and brightly illuminated surface beyond. A sheet of white paper illuminated by a flame will serve. Note the small particles or “threads.” When the gaze is directed at them they appear to move away. Are the “threads” within the eye?

The Limits of Visual Acuity.—Observations on the sense of touch showed that within certain limits two stimuli produce the sensation of a single stimulus, and that these limits vary in extent on different parts of the body. The condition is similar in the eye (E. H. Weber, Ueber d. Raumsinn u. d. Empfindungskreise in d. Haut u. im Auge, Verh. d. k. sächs. Gesell., 1852, p. 145).

Draw on a card two black lines 3 cm. long, 2 mm. wide, and 2 mm. apart. Place the card where it is well illuminated. Retreat from the card until the two lines can no longer be seen as separate lines. Calculate the size of the retinal image of the space between the lines. The diameter of a cone in the fovea centralis lies between 0.004 and 0.005 mm.

*Fix the gaze on a mark on a vertical sheet of white paper, and let the card be moved towards the mark until the two lines can be discriminated. Compare the horizontal and vertical meridia with regard to acuteness of vision in parts outside the fovea.

The Visual Angle and the Test of Normal Visual Acuity.—In estimating the size of the retinal image lines have been drawn from the extremities of the object through the nodal point of the eye to the retina. The angle included between these lines is known as the visual angle. Evidently a small object near the eye and a large object far from the eye may subtend the same angle at the nodal point.

In order to test practically the acuity of vision Snellen suggested the use of letters which at a given distance would subtend at the nodal point a visual angle of 5 minutes. The lines which form the letters and most of the intervals separating the parts of the letters subtend an angle of 1 minute. The letters are arranged on a chart in lines (Snellen. Letterproeven tot bepaling der gezichtscherpe, Utrecht, 1862). Green proposed that the size of the letters increase in geometrical progression from below upwards. Thus a series is provided which, at approximate intervals of 4, 5, 6, 8, 10, 13, 16, 20, 25, 32, 40, and 50 metres, will subtend the same visual angle at the nodal point. The testing distance is 5 metres.

Let the subject sit at a distance of 5 metres from the chart of test letters, which should be uniformly well illuminated. With one eye covered he should with the other endeavor to read the line of letters which at 5 metres subtend the angle taken as the practically “normal” minimum. If he succeeds his vision is said to be normal, and is expressed thus: with V the visual acuity, d the distance from the chart, and D the distance at which the type should be read, $V = \frac{d}{D}$. In this instance $V = \frac{5}{5}$ or 1. The vision is normal.

If at 5 metres the subject can read the line which should be read at 4 metres, he has a visual acuity better than that taken as normal, expressed as $5/4$ or 1.25. Similarly if the smallest letters he can read are those which should be read at 6 metres, his visual acuity would be $5/6$ normal.

Let each student record his visual acuity. Express the visual acuity of a person who reads at 5 metres what he should be able to read at 10, 13, or 16 metres.

Let each student hold before his eyes a +2 D lens. What now is the character of his vision, myopic or hypermetropic? What is the visual acuity under these circumstances? If the unaided eye does not see clearly the line of letters normally seen at 5 metres, but does see them clearly when a concave lens is used, where was the image of the letters formed in the eye? What has been the effect of the lens? Could accommodation make vision normal in this instance?

***Irradiation.** — Associated with the observation of the smallest perceptible image is the phenomenon of irradiation, for it is possible that not only are the sensory endings affected which are directly impinged upon by the light rays, but also neighboring endings as well.

Look at the filament of an electric light with the current turned off. Note its thickness. Now turn on the current. As the filament becomes luminous, is there any apparent change in its thickness? Does this observation suggest a possible defect in the method of determining the smallest perceptible image? How may the projection of the crescent of the new moon beyond the unilluminated part of the moon's disc be explained?

***After-images.** — As in the stimulation of other receptors, so in the eye the excitation outlasts the period of stimulation. The sensation accompanying the persistence of the excited state may be the same as during stimulation or may be the opposite, *i. e.*, the after-image may be positive or negative.

Rest the eyes for two or three minutes by closing them. Then look for an instant at a bright object, such as the filament of an incandescent lamp. Close the eyes again. What is the visual sensation? Is the image bright or dark?

Look at the filament for a half-minute steadily, and then look at a white surface. How does the after-image appear now — bright or dark?

Color Vision

The experiment on chromatic aberration showed that the different spectral rays composing white light have different effects on the retina, giving rise to sensations of different colors. Objects absorb or reflect the rays of white light variously, and the reflected rays thus separated from the rest give color sensations, just as do these rays diffracted by a prism. But not all parts of the eye are capable of responding to the various separated spectral rays (Purkinje, *Neue Beitr. z. Kennt. d. Sehens*, Berlin, 1825, pp. 3-25).

The Field of Color Vision. — Set vertically upright in the frame the blackboard on which are drawn concentric circles whose separation represents an angular distance of 10 degrees. This blackboard will serve as a perimeter. Let the subject place his chin on the rest so that the eye to be tested looks over the top of the upright directly at the centre of the circles, and is 20 cm. distant from the blackboard. The eye should be uniformly fixed on this centre and uniformly accommodated. During the test the other eye should be covered.

Cement to the end of the metal rod a small square of white paper (sides 1.5 cm.), and move it along the outermost arc in the upper median quadrant. If the subject does not see it, repeat the movement on the next arc nearer the centre. After thus finding the arc near the limit of vision, locate by a chalk-mark the point where the paper is first seen as

it is passed inwards along each meridian of the quadrant. Test the lower median and the lateral quadrants in the same way. The area thus outlined represents the visual field of the eye.

Repeat the observation for red, green, and blue colors, using the same size of paper as before. The subject should be ignorant of the color used and should be required to name it as soon as he is certain of its nature. In recording the limits of color vision along the several meridians each color may be represented by its initial letter. Compare the color fields with the field for light. Which color has the smallest field? Is part of the field of vision blind to this color?

Outline in the other eye the field of vision for light. Transfer the results of all the observations to a printed perimeter chart.

Complementary Colors.—When two colors unite to give a sensation of white or gray they are said to be complementary. Thus red is complementary to green, and yellow complementary to blue.

Look at a plate of glass which separates a horizontal yellow surface beneath from a vertical blue surface behind. Let the reflected image of the yellow overlap the blue, seen directly through the glass. What sensation results from the fusion of yellow and blue?

***Inhibition of Color Vision, Retinal Rivalry.**—Look at a sheet of white paper through a piece of yellow glass in front of one eye, and a piece of blue glass in front of the other eye. Continue the gaze for a minute or more. Does the field look yellow or blue or either, at different times, or is there a fusion of the two colors?

Colored After-images.—Colors are not only interestingly related because they may combine to produce a neutral effect, but they show their relations also in after-images.

Look at a flame or incandescent filament through a colored glass for half or three-quarters of a minute. Now look at a white surface. The after-image will appear in the complementary color of the glass. If the glass was red, for instance, the after-image will be greenish.

Examine the perimeter chart again. Were the limits of the field of color vision for red and green closer together than red and blue?

Color Blindness, Holmgren's Test.—The inability to perceive the difference between colors is known as color blindness. The most common form of color blindness is the inability to distinguish between red and green. The simplest method of testing for color blindness is by means of the test-skeins devised by Holmgren (*Meth. z. schnell. Diagnost. d. verschied. Arten v. Farbenblindheit. Upsala Läkaref. Förh.*, 1874, ix, 577). In the following description the method of testing for red-green blindness is alone considered.

Spread out the cloth in which the skeins are wrapped. Select the pale, pure green test-skein and set it near one corner of the cloth. Arrange the other skeins in a well-mixed heap in another part of the cloth. The light should be strong clear daylight. The person examined is expected to pick out 5 or 6 skeins lighter or darker in shade than the test. He must not finger the skeins, but must make his selection and only then pick up the one selected. During the test he should not speak, and persons watching the examination should not make comments or give any other indication as to whether the selection has been appropriate or not. Each student should test himself thus in the presence of a group of other students.

Space Perception

As noted earlier, the eye is the receptor bringing the individual into the widest relations with the world about him. It is the most important and most accurate organ for judging the size of objects and their distance from the body. These judgments of space are based in part on such data as a single eye may give, but the most accurate judgments are made by the use of both eyes in unison.

***Binocular Fusion of Congruous Dissimilar Images.**—On the left side of the note-book draw a dozen light vertical lines about 3 mm. apart and about 2.5 cm. high. Draw a heavy line at the upper and lower ends of the vertical lines. Three cm. to the right of the area covered by vertical lines draw a dark picture of a bird about 1.5 cm. high. Now hold the drawings before the eyes and gaze at them as if looking through the page to a great distance beyond. What change occurs in the position of two separate objects—the bird and the cage?

Binocular Perception of Relief.—With the right eye closed look through a stereoscope at a stereoscopic photograph. Close the left eye and open the right. Compare the appearance of the picture when both eyes are open with its appearance when only one eye is open.

Monocular and Binocular Judgments of Distance.—Draw on the opposite page a circle about the size of a cent. Set the paper at arm's length. Close one eye and try rapidly to place the point of a pencil in the centre of the circle. Compare the result with what happens when the attempt is made with both eyes open, with both eyes shut.

Point the left index finger vertically downward about 18 inches in front of the nose. Look at it with both eyes, then close the left eye and hold the right index finger vertically upward about 6 inches from the nose so as to cut off the view of the lower half of the left finger. With a sudden move, try to strike the left finger with the right. Again compare the result with what happens when the attempt is made with both eyes open and with both eyes shut.

Visual Angle and Apparent Size.—As already noted, the same object subtends at the nodal point a small angle or a large angle according to its distance from the eye. If a familiar object subtends a small angle, what must be the judgment of distance?

With the completion of the study of the sense organs one cycle of bodily activity may be regarded as completed, the cycle concerned in the receiving of stimuli from the surroundings of the body, the transmission of the resulting impulses into the central nervous system, and the discharge of impulses from the central nervous system into outlying muscular structures in such manner that the resulting movements are orderly and adapted to the conditions in the surroundings. All these processes are directly dependent on a supply of food and oxygen. The system by which this supply is continuously maintained is the circulatory system, itself a special example of a muscular mechanism carrying on its functions under nervous control.

THE CIRCULATION OF THE BLOOD

THE function of the circulatory system is the maintenance of a flow of blood through the capillaries, where the interchange of materials between the blood and the tissues takes place. To this end arteries carry the blood to the capillaries, and veins carry the blood away from them. The force that drives the blood through arteries, capillaries, and veins is the contraction of a portion of the vascular system with greatly thickened muscular walls — the heart.

CARDIAC CONTRACTION AND ITS CONTROL

The main features of cardiac contraction can be studied as well on the amphibian heart as on the mammalian. Indeed, many of the characteristics of the heart beat were discovered first in the frog and the tortoise and later proved true also of warm-blooded animals.

*Experiments on the Frog Heart

The Heart is Automatically Rhythmic. — Expose the heart of a frog as described on p. 16. Cut widely around the sinus venosus and the veins entering it, sever the two aortae, and thus separate the heart from the body. Place the excised heart in a watch glass. Does the contraction continue?

If so, note the rate immediately after the excision and again a few moments later. What is the relation of cardiac contraction to the central nervous system?

The Automaticity of Parts of the Heart. — Cut between the sinus and the right auricle at the sino-auricular junction. Wait a few moments after the operation. Does the sinus continue to beat?

Does the rest of the heart continue to beat? If so, what is the rate of the beat?

Cut between the auricles and the ventricle just above the auriculo-ventricular groove. Wait for possible effects of the operation to pass away. Do these parts contract when separated? If so, note the rate of contraction.

Cut through the ventricle just below the auriculo-ventricular groove. Does the apex of the heart beat spontaneously? Stimulate it mechanically by a gentle tap. Stimulate it chemically by laying on it a crystal of sodium chloride.

Is the apex irritable? Does the constant chemical stimulus cause periodic contractions?

Changes of Irritability during a Single Cycle. — Expose the heart of a frog. Tie a fine wire through the tip of the ventricle and another fine wire into the base of the ventricle. Fasten the other ends in the posts of the secondary coil of the inductorium. Introduce the wires only into the muscular wall of the heart; be careful to avoid loss of blood. By means of a silk thread connect the tip of the ventricle with the heart lever. Let a signal, set *as close as possible beneath the heart lever*, record the moment of stimulation. Start the drum at a rate which results

in a widely separated record of systole and diastole. Study the record with reference to the contraction. Is the contraction of the auricle shown in the tracing?

Stimulate the ventricle with minimal make or break shocks, but do not let the two stimuli fall within a single cardiac cycle. Try to stimulate at various stages in systole and diastole, but let normal beats intervene between those which are disturbed. By means of simultaneous ordinates determine the moment when the stimulus was applied.

What resulted from the stimuli applied during systole?

What from the stimuli applied during diastole?

Explain how a constant stimulus normally causes a rhythmic beat.

During systole the heart is said to be "refractory" to stimulation. Even after systole there is only a gradual restoration of maximum irritability.

Was the "extra contraction" followed by a longer pause than usual?

Note the relation of any such "compensatory pause" to the auricular contraction. Does the next ventricular contraction resume the normal rhythm? (Marey, *Travaux du labor.*, 1876, ii, 63.)

The Passage of the Contraction Wave.—The rheoscopic nerve-muscle preparation (p. 30) showed that preceding each heart beat there is an excitatory change in the muscle. This change sweeps over the heart as a wave, and the contraction that follows also sweeps over the heart as a wave, from the sinus, or the ends of the great veins, through the auricles, to the ventricles. There is controversy as to whether this wave is conducted by the branching cardiac muscle fibres or by the nerve fibres in the muscular wall. The following experiments have been cited in favor of muscular conduction.

Gaskell's Clamp and Heart Block.—The contraction wave does not move at the same speed throughout its course. Between the auricular contraction and the ventricular contraction there is a pause, called the auriculo-ventricular interval. This interval has been attributed to the relatively small number of muscular fibres connecting the auricles and the ventricles, and to the embryonic character of these fibres. Compression of this region is supposed to reduce still further the number of fibres capable of conveying the wave of contraction (Gaskell, *Phil. Trans. Royal Soc., London*, Pt. iii, 1882, p. 996).

Expose the beating heart of a frog with brain pithed. Draw the ventricle between the edges of the Gaskell clamp. Screw the edges carefully against the auriculo-ventricular junction. Continue to increase the pressure very cautiously. A degree of pressure will be found at which the ventricle does not respond to every beat of the auricle, *i. e.*, the contraction wave does not always pass from auricle to ventricle through the partial block.

What numerical ratios are noted between the contractions of the auricles and the contractions of the ventricle, as the pressure is slowly increased, and again as the pressure is lessened?

Engelmann's Incisions.—With small scissors cut about three fourths across the ventricle, alternately on the two sides, beginning just below the auriculo-ventricular junction. Thus the ventricle is transformed into a zig-zag strip. If the contraction wave is still conducted into the ventricle, note whether the wave passes over the strip. If no part of the ventricle is active, stimulate the base with a minimal induction shock. Next apply the same stimulus to the apical end of the strip. The wave of contraction should travel equally well in either direction. (Engelmann, *Arch. f. d. ges. Physiol.*, 1875, xi, 466.)

(*Demonstration. — The Current of Action of the Heart.* — Previous experiments have shown that contraction of skeletal muscle is accompanied by the development of electrical potential. The electromotive phenomena of the heart beat can be shown not only in the exposed heart, but in the intact body.

Expose the heart of a frog in the manner already described. By means of filter paper remove any free fluid there may be about it. To the clay points of the two non-polarizable electrodes attach short strings wet with isotonic salt solution and bring together the two strings. Connect the electrodes to a capillary electrometer or a thread galvanometer and make sure that they are iso-electric. Now separate the strings and let the end of one touch the base of the heart, the end of the other the apex. Bring the heart into connection with the recording instrument. What occurs?

What inference may be drawn as to the nature of the stimulus affecting the nerve laid on the muscle?

The difference of potential arising just before the muscle contracts produces the "action current" already seen in the normal contraction of skeletal muscle. It has been shown that with each heart beat a wave of potential-difference sweeps through the human body; by connecting points on the skin lying on opposite sides of the equator of the heart an action current can be demonstrated in the capillary electrometer (Waller, *Phil. Trans. Royal Soc.*, 1889, clxxx B, 169). A photograph of the electrometer movements is called an "electrocardiogram.")

The Influence of Ether and Chloroform. — Excise two frogs' hearts completely, and place each one in a watch glass with about 5 c.c. of Ringer's solution. To one add a drop of pure chloroform, and cover with another watch glass. Note the time until the heart stops beating.

Repeat on the other heart the same procedure, but use ether instead of chloroform. How do these two anaesthetics differ in their effect on the heart?

Inhibition of the Heart Beat. — Experiments already performed have proved that the heart is automatically rhythmic. The beat, however, can be made faster or slower, according to the needs of the body, by nervous impulses coming to the heart from the central nervous system. Three sets of nerves connect the central nervous system with the heart: the vagi, which check the contraction; the sympathetic nerves, which augment and accelerate the contraction; and the depressor nerves, afferent to the medulla, which regulate the resistance in the blood vessels according to the needs of the heart. The first two sets of nerves, controlling the rate and force of the heart beat, will be considered now; the depressor nerves will be studied in connection with the innervation of the blood vessels.

The observation that stimulation of the vagus nerve stops the heart is of historic interest, for it was this observation by the brothers Weber, in 1845, that first established the fact that nervous impulses can inhibit as well as cause activity. This important discovery was made on the frog (Ed. Weber, *Wagner's Handwörterbuch d. Physiol.*, 1846, iii (2), p. 45).

Inhibition of the Heart by Vagus Stimulation. — Arrange the heart to record its contractions clearly on a slowly revolving drum. Set in the primary circuit of an inductorium a simple key and an electromagnetic signal. Using a weak interrupted current, stimulate

the vagus nerve; first for 5 seconds with the weakest current producing any effect, next with a strong current for the same length of time, and finally with the strong current for

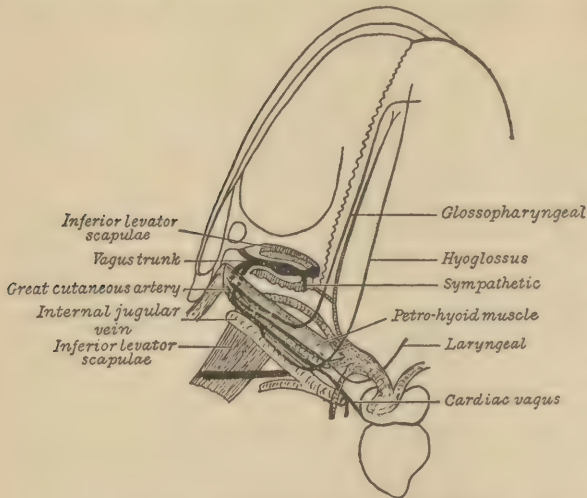


FIG. 9. — Relations of vagus nerve in frog, ventral aspect. Part of the lower jaw has been removed and the remnant moved to the right; the mucous membrane covering the roof of the mouth has also been removed, thus exposing the inferior levator scapulae muscle. By severing this muscle near its origin the vagus trunk and the connection with the sympathetic have been uncovered.

Dissection of the Vagus Nerve in the Frog. — Pith the brain of a frog and expose the heart in the manner already described. Fasten the fore limbs in the frog holder so as to pull widely apart the two portions of the pectoral girdle. Arising from the angle of the jaw and passing inwards and backwards are the ribbon-like petro-hyoid muscles. Two nerves pass forwards across each muscle; the glossopharyngeal, lateral, running to the muscles of the floor of the mouth, and the hypoglossal, nearer the median line, also going forward to the tongue. Two other nerves will be found lying along the superior vena cava just below the lower border of the petro-hyoid muscle. These are the cardiac and laryngeal branches of the vagus. The larynx passes inwards and forwards towards the larynx.

Separate the cardiac branch of the vagus by means of a blunt dissector and let the nerve near the angle of the jaw rest on the tip of a glass rod. Sever the other three nerves at some point in their course. Enough of the vagus should be free to permit electrodes to be applied to it. Keep the nerve moist with isotonic salt solution.

a longer time. The signal should record directly below the writing point of the heart lever.

Study carefully the records. How many heart beats are included in the latent period? Is the heart arrested in systole or in diastole?

Does the record indicate any change in the tonus of the heart during the stimulation?

How long does the effect last after the stimulation has ceased?

Is the beat resumed with full vigor or is there a gradual increase in the vigor of the beat?

Is the rate of contraction changed after stimulation?

How does the heart act under prolonged stimulation of the vagus?

The Irritability of the Inhibited Heart. — While the heart is stopped in vagus inhibition, pinch the ventricle with small forceps. If the inhibition is not deep, the ventricle should respond; but with profound inhibition the stimulus may be without effect.

Intracardiac Inhibition. — While the heart is recording its contractions, bring the electrodes in contact with the white crescent at the sino-auricular junction, and stimulate with a weak interrupted current. Record the effect.

The Action of Nicotine. — The stopping of the heart when the crescent is stimulated might be due to excitation of vagus fibres, or it might be due to excitation of other inhibitory nerves through which the vagus impulses normally act. In studying the sympathetic nervous system Langley found that nicotine does not affect nerve trunks, but when applied to outlying ganglia it blocks impulses at the synapse between pre-ganglionic and post-ganglionic neurons. If the vagus acts through nerve cells in the heart, nicotine can be used to block the impulses at the synapse between the vagus fibres and these cells.

Stimulate the vagus nerve and make sure that it stops the heart. With a camel's hair brush paint the heart with a 0.2 per cent solution of nicotine. After three minutes stimulate the vagus as before. Is the heart now inhibited?

If it is, apply the nicotine solution again and repeat the test.

If the heart is not inhibited, stimulate the crescent between the sinus and right auricle. Do the vagus fibres pass directly to the cardiac muscle, or do they act through nerve cells lying in the heart?

Pilocarpine Inhibition and Atropine Block.—Apply to the same heart washed with isotonic salt solution, or to a freshly prepared heart, a 1 per cent solution of pilocarpine. How is the tone of the heart muscle, and the frequency and amplitude of the heart beat affected by the drug?

Wash with isotonic salt solution the heart already affected by pilocarpine, and apply a 0.5 per cent solution of atropine sulphate. What now happens to the tone of the heart muscle and to its rhythmicity?

Stimulate the vagus nerve. Is the heart stopped?

Stimulate the crescent. Is the heart stopped by this procedure?

Atropine abolishes the efficiency of post-ganglionic fibres.

The Centre for Cardiac Inhibition.—The foregoing experiments have proved that the inhibition of the heart is a peripheral effect caused by impulses coming to the heart by the vagus nerve. These impulses originate in the central nervous system, probably as a reflex response.

Etherize a frog and beginning at the lower end of the spinal column carefully lay bare the central nervous system by the method used in the examination of the functions of the dorsal and ventral roots (see p. 36). Expose the heart. If the animal shows any signs of recovery from the anaesthetic, administer more ether.

Suspend the frog by a hook passed through the lower jaw. Stimulate various regions of the spinal cord with a weak interrupted current. Stimulate the cerebral hemispheres. Is the heart affected?

Apply the stimulus to the medulla. Is the heart now inhibited?

Where is the centre for cardiac inhibition?

If the animal is not used for the next experiment, be sure to destroy the brain as soon as the position of the cardiac inhibitory centre is found.

Reflex Inhibition of the Heart.—Pith the cerebrum of a frog, with care not to injure the medulla, and lay bare the pericardium. In these operations make small incisions and handle the parts cautiously. Note the rate of the heart beat. Strike the abdomen sharply and repeatedly with the handle of the scalpel.

The heart should beat more slowly or be temporarily arrested in diastole. When this result has been secured pith the entire central nervous system of the frog, and repeat the experiment. Explain the result. (Goltz, Virchow's Arch., 1863, xxvi, 10.)

Augmentation of the Heart Beat.—The nerves causing augmentation and acceleration of the heart rate, as already stated, come to the heart from the sympathetic chain. In mam-

mals they have a course separate from the vagus, but in the frog the vagus trunk carries the augmentor fibres as well as the inhibitory. These two sets of fibres can be separated, however, in the frog, as Gaskell has shown (*J. Physiol.*, 1884, v, p. xiii).

Stimulation of the Sympathetic in the Frog.—Expose the anterior end of the sympathetic chain by the method described in the experiment on the innervation of the iris (see p. 47). The pre-ganglionic fibres of the frog's cardiac sympathetic supply arise from the third spinal nerve. The cell bodies of the post-ganglionic neurons probably lie in the third sympathetic ganglion. Pass under the sympathetic cord opposite the large brachial nerve (the second spinal) a fine silk thread. Tie the thread and sever the nerve below the ligature.

Prepare to record the beat of the heart and the period of stimulation as in the experiments on the action of the vagus nerve. If the heart is beating rapidly, reduce the rate by applying cold isotonic salt solution. While the heart beat is being distinctly recorded on a slowly revolving drum, momentarily close the key in the primary circuit. Close it again in 20 seconds, but this time an assistant should have the platinum electrodes in contact with the sympathetic cord. Keep the key closed for 20 seconds and stimulate during this period with an interrupted current of moderate strength. When 20 seconds more have elapsed after the stimulation has ceased, momentarily close the key again, and continue thus recording 20-second intervals for 6 or 7 periods.

Count the rate for each period. What change of rate does the stimulation produce? Is the force of the contractions changed? Compare the latent period and the after-effect of stimulation of the sympathetic with the same results of vagus stimulation.

It has already been proved that a 0.2 per cent solution of nicotine painted on the heart will stop vagus impulses from reaching the post-ganglionic fibres in the heart. The post-ganglionic fibres of the sympathetic, however, are not in the heart, but in the vagus trunk. By applying the nicotine solution to the heart, therefore, it is possible to block the vagus impulses and permit the augmentor effects to appear. Perform this experiment, using the method of recording described in connection with stimulation of the sympathetic strand, and stimulating the vagus trunk.

Experiments on the Tortoise Heart

The tortoise heart is much larger and stronger, its relations are simpler (especially its innervation), and it shows certain features of cardiac contraction more clearly than the frog heart. For these reasons it is preferable to the frog heart as a means of demonstrating the basal facts of cardiac physiology. The experiments in this section can all be performed, in the order given, on one tortoise and in a single laboratory exercise. Before starting the experiment, read through the entire description. Since frequent reference will be made to the experiences in this section, be sure that every point is understood. Submit the records to an instructor for discussion before undertaking further experiments.

A turtle is supplied which has been pithed. Remove the plastron. Slit the pericardium and expose the heart. Identify the different parts and trace the sequence of the contraction wave from the sinus to the bulbus arteriosus.

1. Inhibition by Vagus Stimulation.—Expose the vagus nerves at the base of the neck and place silk ligatures loosely about them. The ligatures will be tied later. Arrange an

induction coil for tetanizing currents of moderate strength, and, using the briefest possible periods of stimulation, make sure by physiological test that the nerves isolated are the vagi.

(a) Describe the appearance of the heart during vagal stimulation.

Arrange two light heart levers to write on a smoked drum; set the writing points in the same vertical line, and directly beneath them place an electro-magnetic signal connected with the clock. By means of silk ligatures connect the two auricular tips with the upper lever, and the tip of the ventricle with the lower lever.

(b) While recording simultaneously the contractions of the auricles and ventricle, carry out the following procedures. Be sure to leave a sufficient interval of time after each step to permit a return to the former state.

Stimulate the left vagus.

Stimulate the right vagus.

Tie the ligature on the left vagus.

Tie the ligature on the right vagus.

Apply prolonged stimulation to the right vagus peripheral to the ligature.

Describe the results in detail.

2. Heart Block. — Without disarranging the apparatus, place the modified Gaskell clamp so that, on tightening, the narrow groove between the auricle and ventricle will be compressed. While recording simultaneously the contractions of the auricles and the ventricle, make the following experiments.

(a) Gradually close the clamp until partial heart block is produced as indicated by failure of the ventricle to follow the auricles, and while it exists stimulate first the left vagus and then the right vagus.

(b) Gradually close the clamp so as to produce complete block and then release the clamp. Do this carefully and avoid going so far as to injure the tissue. Ventricular cessation indicates a sufficient degree of pressure.

3. Cardiac tonus. — While the heart is still in place, cut the two auricles, with the sinus attached, away from the ventricle. Leave in place the ligatures about the tips of the auricles, attaching one to an L-shaped glass rod, the other to a heart lever. Slit open the auricles with scissors in order to promote rapid diffusion of salts. Set the glass rod and the auricular muscle in a beaker lined and almost covered with moistened filter paper. Let the drum turn at a speed which just fails to cause a fusion of the records of the auricular contractions. Note that after a time the separate beats are superposed on oscillations in the base line. These are the tonus oscillations. Do the tonus curves recur rhythmically? Is there any alteration of the auricular beats as the tonus rises? (Fano, *Beitr. z. Physiol. Ludwig's Festschrift*, 1887, p. 287.)

Place a drop of dilute solution of adrenalin on the beating preparation. Explain the result.

4. The Influence of Inorganic Salts. — The whole heart, and parts of the heart, can be kept in activity by the application of proper solutions of the inorganic salts of the blood. It is quite possible that the contraction of cardiac muscle is normally maintained by the balanced action of these salts. The effects of the salts individually and in combination are seen in the following experiments. (See Howell, *Am. Journ. Physiol.*, 1901, vi, 181.)

Fill the beaker used in experiment 3 with 0.75 per cent NaCl plus 0.01 per cent NaHCO₃,

immerse the strip, and place beneath the beaker the wooden stand. Allow oxygen to bubble through the solution. At intervals of 10 minutes record a series of the contractions. As time elapses is there any change in the height of the contractions?

Is there any change in rate?

Is there any alteration of the tonus?

When the strip shows signs of exhaustion add to the solution 1 c.c. of 1 per cent CaCl_2 . Is there any change in the activity?

If the activity is well restored by the CaCl_2 , replace the contents of the beaker with 50 c.c. of 0.7 per cent NaCl containing 20 drops of 0.9 per cent KCl . What is the effect of potassium?

As soon as the characteristic effect of potassium has occurred, replace the KCl with Ringer's solution. This solution should maintain prolonged rhythmic contractions.

5. The Influence of Changes of Temperature.—Replace the solution in which the strip is contracting by Ringer's solution cooled to 10°C . How is the rate of beating affected by cold?

Replace the cold salt solution with Ringer's solution at 30°C . What is the rate of beat under this condition?

Compare these results with previous observations on skeletal muscle and nerve.

(Demonstration. — The Exposed Mammalian Heart.—Under urethane anaesthesia (2 gm. per kilo by stomach) and artificial respiration cut an oval opening from the front of a cat's thorax between the uppermost and the lowest costal attachments to the sternum. The opening, which should be but slightly larger than the heart, reveals that organ beating within the pericardium. Slit the pericardium along the midline, and attach the edges to the edges of the thoracic window by three strong sutures on either side and one at each end. Then by continuous suture sew the thoracic and pericardial edges together so as to close tightly the opening into the pleural cavity. Any air that may be in the cavity should be withdrawn through a hollow needle. Then artificial respiration may be discontinued. (See Drinker, J. Exp. Med., 1921, xxxiii, p. 675.)

Each vagus nerve is stimulated and the effect on the heart noted. Adrenalin (0.5 c.c.: 1:100,000) is injected into the left ventricular cavity and the consequences observed. The animal is made to breathe a mixture of illuminating gas and air, until heart block occurs. The heart is allowed to recover. By use of proper levers the contractions of auricles and ventricles may be recorded simultaneously in the foregoing procedures.)

(Demonstration. — The Nutrition of the Heart through the Vessels of Thebesius.—The cat in the foregoing demonstration is bled from the carotid artery. As the blood flows it is defibrinated. When the bleeding has almost ceased the heart is removed and placed in Ringer's solution (mammalian) at 38°C ., in which it pumps itself clear of blood. A glass tube with a slight constriction near the end to receive a thread is now tied into the right ventricle by a ligature at the auriculo-ventricular groove. A mixture of Ringer's solution (at 38°C .) and the defibrinated blood is poured into the tube, and the heart is supported by the tube in a beaker of warm Ringer's solution. The excised mammalian heart will, under these conditions, exhibit rhythmical contractions for a long period. The wall of the right ventricle has numerous openings of the Thebesian vessels. Pratt proved that the

heart in the conditions of this experiment was nourished through these vessels (*Am. J. Physiol.*, 1898, i, 86).

The heart is surrounded with warm and then with cold Ringer's solution, and the effects of the temperature change are recorded. An induced current is applied, in single shocks and also rapidly interrupted, and the results observed.)

THE FLOW AND PRESSURE OF THE BLOOD

The study of the characteristics of the heart muscle and its control by extrinsic nerves affords a basis for the further study of the flow of the blood in the vessels leading from the heart and back to the heart again. In this system of vessels, in which the blood circulates, the flow manifests peculiarities of rate and pressure fairly typical for each division of the system — the arteries, capillaries, and veins. Some of these peculiarities can be seen in the frog's tongue.

The Circulation seen under a Microscope. — A frog will be provided which has been anaesthetized with urethane (0.03 c.c. of 10 per cent urethane per gm. of frog, injected into a lymph sac one hour before). With the animal lying on his abdomen, open the mouth, draw out the tongue and pin it over the hole in the frog board. Do not stretch the tongue more than is necessary to make it translucent. As a rule use the low power objective of the microscope, but for particular study turn to the high dry objective. Select a field where blood is flowing in vessels of different sizes, and by means of clips on the microscope stand fix the frog board in place. Keep this field in view as long as reactions occur satisfactorily. The preparation must be kept moist with cold-blooded Ringer's solution, but the fluid should not be excessive when the high-power objective is employed.

Note the larger vessels and the smaller richly branched vessels. Note the two kinds of larger vessels, one in which there is pulsation, the other without pulsations. What is the direction of flow in the pulsating vessels, away from the body or towards it?

Study the relations between the flow in the larger and the smaller vessels. Does the blood pass from the pulsating vessels into the smaller, or vice versa?

The smaller vessels are the capillaries. Of the larger, which are arteries and which are veins?

From observation of the direction in which the blood flows, what inference may be drawn as to the relative pressure of the blood in arteries, capillaries, and veins?

What characteristic alteration of pressure occurs in the arteries that does not appear in the capillaries and veins?

Note carefully the relative speed of the flow in arteries, capillaries, and veins. Where is the rate slowest?

Observe that although the direction of the flow indicates a greater pressure in the capillaries than in the veins, the rate of flow is greater in the veins than in the capillaries.

Examine the tongue with the high-power objective. Study the character of the flow in a small artery or vein. Is there any difference between the speed of movement of the corpuscles in the middle of the stream, and along the vessel wall? Compare the flow in the larger vessels with the flow in the capillaries. Find a white corpuscle and watch its course. With patience a white corpuscle can be found making its way by amoeboid movement through the capillary wall.

Conditions Affecting the Capillary Circulation. — After the foregoing study of the circulation as seen under the microscope, choose a field which includes several capillaries and the arterioles from which they lead. Capillary changes can be seen best in short branches or shunts which connect one vessel with another.

(1) Make a rapid "single line" sketch of the field or part of the field, identifying capillaries, veins, and arterioles. Note differences in blood flow in different vessels and in the same vessel at different times. Observe the plasticity of the corpuscles, which can be seen best by watching with the high dry objective a narrowed lumen where a capillary branches from an arteriole.

The average size of a red blood corpuscle of a frog is 22μ long, 15μ broad, and 4μ thick. What do you estimate is the average diameter and length of a frog's capillary expressed in millimetres?

If there have been any changes in the number of active capillaries, record your observations.

(2) Make a sketch whenever a different field is studied. Stimulate a capillary area by lightly stroking the surface of the tongue with a cat hair held in forceps, while keeping the field under observation. Vary the amount of pressure used. Record any observations, noting the latent period and the duration of the reaction, if any is seen.

(3) Using a fine pipette, apply a small drop of 10 per cent urethane to a definite area under observation. Record the results by a sketch and description.

Repeat, using 0.1 per cent adrenalin. In applying a new solution always select a new area or wash away the previous reagent with Ringer's solution. Allow time for subsequent drying, to avoid too great dilution of the next fluid applied.

(4) Without disturbing the preparation expose the tongue to a draught of cold air from an open window or by fanning. Record any changes that are observed.

(5) Make a rapid sketch of the field. Cut off the circulation to the tongue by gently pressing near the mouth with the edge of a microscopic slide. Sketch the field again. Give an account of the appearance of the field during vascular occlusion and after circulation has been restored. (For an account of the original observations on which these exercises are based, see Krogh, *J. Physiol.*, 1920, liii, p. 399.)

The Capillary Circulation in Man. — Scrub the back of the hand and the base of the nails with a brush and soap. At the base of one of the nails place a drop of paraffine oil. Using direct illumination, either bright sunlight or light concentrated from a small tungsten spiral, examine, with a 16 mm. or lower objective, the capillaries in the oiled area. Note the arrangement of the loops, and their variable size and shape. Produce congestion by constricting the base of the finger or wrist with a string or rubber band tight enough to prevent venous flow. What is the effect upon the diameter of the capillaries and upon the number visible.

Repeat the examination after plunging the hand into ice water, and after warm water (42° – 44° C.).

With the sharp pressure of a toothpick or finger nail, draw a line on the skin being examined. Compare the condition of the capillaries in the red streak with those adjacent.

If possible examine the margins of a healing abrasion and note the capillary arrangement. (Lombard, *Am. J. Physiol.*, 1912, xxix, p. 335.)

As stated in the first paragraph of this study of the circulation, the function of the circulatory system is the maintenance of a flow of blood through the capillaries. As the foregoing observations have shown, the walls of the capillaries are extremely thin. Outside the capillaries, bathing all the tissues, is the lymph. The lymph is formed by the passage of the fluid portion of the blood and some of the white corpuscles through the capillary walls. Through the capillary walls, also, oxygen and food materials pass from the blood to the lymph and thence to the tissues; and from the tissues carbon dioxide and other waste products pass to the lymph and thence through the capillary walls to the blood stream, by which they are borne to the organs of excretion. The lymph which escapes from the blood in the capillary region flows away from its place of origin, carrying waste, and is gathered at length into lymph vessels which pour the lymph back to the blood again in veins near the heart. Thus the capillary region is of the greatest importance not only for the nutrition of the body, but also for the riddance of waste. What advantage, therefore, lies in the flow of the blood being slower in this region than in other parts of the circulatory system?

The relatively slow flow in the capillary region is not due to greater friction in this region because the vessels are smaller, for in that case the flow would be still slower in the veins, where additional friction is encountered. The alterations of the rate of flow in the three classes of vessels are dependent on the relative total area of cross-section of these vessels. Thus the total cross-sectional area of the capillaries is estimated as normally about 700 times that of the aorta near the heart. On the other hand, the aorta is not much smaller than the veins emptying into the right auricle. Since, in order to maintain a circulation, an equal quantity of blood must pass through arteries, capillaries, and veins, at the same time, it is evident that in the fulfilment of this condition the blood must flow much faster where the cross-section is small than where it is large. The rate therefore becomes gradually less in the arteries, and is least in the capillaries; in the veins the rate begins to increase again and near the heart is almost as fast in the veins as it is in the aorta. How is it possible that, although the pressure in the veins is less than in the capillaries, the velocity of the blood is greater?

The Events of a Cardiac Cycle

Between the beginning of one cardiac contraction and the next there must be for a time a higher pressure in the ventricle than in the aorta, otherwise the contents of the ventricle would not be forced into the aorta; and during the remainder of the cycle, while the ventricle is in relaxation, the pressure in the ventricle must be not only less than that in the aorta, but also less than that in the auricle, for otherwise the auricular contents would not enter the ventricle. In order that these changes of relative pressure may prevail it is necessary that during contraction the ventricular pressure be supported by the mitral valve, and during relaxation the aortic pressure be supported by the semilunar valves.

***The Action of the Valves in the Mammalian Heart.**—Open the pericardium of the sheep's heart; note its attachment to the great vessels. Identify the pulmonary artery and veins, the aorta, and the venae cavae. If the trachea and lungs are present, remove them without injury to the heart.

Wash out any clots that may be in the ventricles. Tie a short glass tube (10 cm.

long) into the superior vena cava. Tie a longer tube (50 cm.), curved at one end, into the pulmonary artery. By means of a short piece of rubber tubing connect the short glass tube with a funnel held in a funnel ring. Support the longer glass tube vertically, with the curved end over the funnel. Ligature the inferior vena cava and any other vessels that may leak. Let the heart be suspended over the sink in the desk. Pour water into the funnel until the level rises into the funnel. Compress the right ventricle. In which tube does the water rise? Explain. Release the ventricle. Does the water fall in the long tube? Explain. Alternately compress and release the ventricle, pouring more water into the funnel if necessary, until a circulation is established.

Remove the long tube and cut one of the semilunar valves. Replace the tube, and again rhythmically compress the ventricle. The water level is not maintained in the tube, for the valve is "incompetent," and the water pumped out regurgitates into the heart. Compress the heart more vigorously and more rapidly and attempt to establish a "compensation" for the injured valve. Remove the two glass tubes and tie the pulmonary artery. Remove most of the right auricle. Let water run between the auriculo-ventricular valves into the ventricle. The segments of the valves float up and close the orifice. Invert the heart. Are the valves competent? Cut the chordae tendinae and again let water run into the ventricle. Are the valves now competent? Open the right ventricle. Draw a diagram showing the insertion of the chordae tendinae into the valves.

Separate the aorta from the left ventricle, taking care to dissect widely enough to avoid injuring the semilunar valves. Ligature the coronary arteries. Tie a tube into the distal end of the vessel, and pour water into it. Are the aortic valves competent?

Beginning at the apex of the heart make transverse sections until both ventricles are opened. Note the relative thickness of the walls. With what is this related in the work of the heart? Examine the veins opening into the auricles; are they provided with valves? Measure the diameter and compare the sectional areas of the two venae cavae, the pulmonary artery, and the aorta. Compare the elasticity of the walls of these four vessels. Slit open the aorta between two of the semilunar valves. Note the pockets behind the valves; note how the valves are related to each other and how they are attached to the wall. Feel the small nodule in the free edge of each valve. Open the right ventricle and examine the inner surface for the openings of the vessels of Thebesius.

Examine carefully the base of the interauricular septum on the right side, and note the coronary sinus. Below and to the right of the sinus lies the "auriculo-ventricular node," a group of undifferentiated muscle fibres, which can be distinguished, after tearing aside the endocardium with a needle, by a color paler than the other cardiac muscle. Passing downward from the node is a ribbon of this paler muscle, the main bundle of His. Follow the ribbon to where it divides into the right and left septal branches, and farther, if possible, to the papillary muscles situated on the septum. (Kent, J. *Physiol.*, 1893, xiv, 241; His, *Abh. d. k. sächs. Gesell. d. Wiss., math.-phys. Kl.*, 1893, xix, 1.)

(*Demonstration.* — *The Action of the Valves in the Ox Heart; Gad's Method.* — Brass cylinders, holding glass windows, are tied into the left auricle and into the aorta. Into the wall of the ventricle a test-tube is fastened so that it projects into the ventricular cavity. Through the apex there is introduced into the cavity a tube connected with a large rubber bulb. A rubber tube leading from the base of a pressure bottle is attached to the cylinder tied into the auricle, and another rubber tube is arranged between the cylinder in the

aorta and the top of the pressure bottle. The pressure bottle is now filled with water. The water passes down the tube to the auricle, fills the auricle, the ventricle, the tube leading to the bulb, the bulb, and the tube rising from the aorta. Into the test-tube a small electric lamp is introduced; the interior of the heart is illuminated. As the bulb is pressed ventricular pressure rises, the mitral valves close, and water pours out into the aorta. As the pressure on the bulb is released ventricular pressure falls, the semilunar valves close, the mitral valves open, and water pours into the ventricle. The illumination of the interior of the ventricular cavity permits the action of the valves in response to pressure changes on either side of them to be clearly seen.)

The Heart Sounds.— Let the subject lay bare the chest. If the ribs are not too much covered with fat a gentle heaving of the chest wall can be observed in the fifth left intercostal space slightly within the nipple line. This is the “cardiac impulse.” Feel it. Note how it changes as the subject changes from a standing posture to lying horizontal on the left side, and on the right side.

After cleaning the ear-tubes of the stethoscope, place them with the tips pointing inward and upward, in the ears. Set the bell or the disc of the instrument over the region of the cardiac impulse. During the examination the parts of the stethoscope should not rub against anything, or be breathed upon, and there should be no talking.

Observe the two sounds. While listening, note the relation of the sounds to the cardiac impulse or to the pulse felt in the carotid or brachial artery. The sound heard just at the beginning of the pulsation is called the first sound, the one that follows is the second sound. The first is caused by the contraction of the cardiac muscle, and in part also by the closure of the auriculo-ventricular valves, and possibly by the vibrations of the chordae tendinae. The second sound is due to the sudden closure of the semilunar valves in the aorta and in the pulmonary artery. (Williams, *Rep. Brit. Assn.*, 1836, p. 269.) Compare the two sounds in duration, pitch, loudness, and quality.

Place the stethoscope over the second right costal cartilage, which lies over the arch of the aorta, and listen again. Record any change that is noticed in the relative loudness of the two sounds. Is there any change in the quality of the first sound?

It is important for the student of medicine to become well acquainted with the normal character of the heart sounds, for when the valves are injured the sounds are typically altered, and by means of these altered sounds the position of the lesion can be judged. It is especially important to be able to recognize the interval between the first and second sound, the systolic interval; and that between the second and the first sound of the next cycle, the diastolic interval.

***Cardio-Pneumatic Movements.**— Light a fish-tail gas burner, and turn the flame low. Hold in the lips a long glass pipette. With one hand direct the narrowed end of the pipette near the flame, with the other hand close the nostrils. Suspend respiration, but keep the glottis open. What rhythmical disturbance is seen in the gas flame?

Are the movements synchronous with the heart beats?

Offer an explanation of the phenomenon.

The Nervous Control of the Peripheral Resistance

Arterial pressure is determined by the inflow into the arterial system, which is largely dependent on the heart rate, and by the resistance to the outflow from the system. The study of the innervation of the heart has already shown the nervous control of one of the factors concerned in arterial pressure; it is important to know the nervous control of the other factor — the peripheral resistance. The resistance is to a large extent dependent on the tonus of the smooth muscle in the walls of the arterioles. This tonus is in turn normally dependent on the tonic activity of the vasoconstrictor neurons. Through these neurons the contraction of the vessels can be increased or diminished and the peripheral resistance correspondingly altered.

Vasoconstriction from Stimulation of the Sciatic Nerve. — Anaesthetize a frog with urethane (0.03 c.c. of 10 per cent per gm. of body weight), and then curarize the animal very lightly (large doses of curare paralyze the vasoconstrictor mechanism). Lay bare the sciatic nerve, and isolate it, with greatest care not to injure the accompanying blood vessels. Pass a thread under the nerve. Place the frog, ventral side down, on the small frog-board with the foot of the operated leg over the V-shaped opening. Spread the web gently over this opening, and hold it in place with pins. If the web is drawn too tightly the blood will not flow through it. Cover the frog with wet filter paper, and keep the stretched web moist on both surfaces.

After finding a field which shows clearly arteries, capillaries, and veins, tie the thread tightly around the sciatic nerve, and cut the nerve central to the ligature. Is any change noted in the rapidity of the flow?

With an interrupted current stimulate the nerve distal to the ligature. What change occurs in the circulating blood?

How may this change be explained?

(Demonstration. — Vasoconstriction by Stimulation of the Cervical Sympathetic Nerve. — A rabbit with a white ear is anaesthetized with urethane, and anaesthesia is maintained until death. After tracheotomy the cervical sympathetic nerve is exposed below the white ear. A thread is tied around the nerve and the nerve is cut below the thread.

Compare the appearance of the two ears. The ear deprived of its sympathetic innervation feels warmer and its blood vessels are more dilated. The sympathetic neurons were evidently exercising a tonic effect.

Hold before a bright light the ear in which the vessels are dilated. With an interrupted current stimulate the cervical sympathetic above the ligature. What change appears in the blood vessels of the ear?

Is there a latent period?

Does the effect outlast the stimulus?

Wrap the ear about a thermometer and repeat the stimulation. Is there any change in the temperature as vasodilation is replaced by vasoconstriction? (Bernard, *Compt. rend. Acad. des Sci., Paris, 1852, xxxiv, 472.*)

What would happen if the superior cervical ganglion were painted with a weak solution of nicotine and the stimulus were again applied below the ganglion?


How could the vasoconstrictors to the small intestine be demonstrated?

***The Vasomotor Function of the Spinal Cord.**—Etherize a frog and expose the heart without loss of blood. Also expose the intestine by a longitudinal incision on one side of the median line. Suspend the frog from a hook. Carefully note the distention of the heart and the aortae. Note also the vascular conditions in the mesentery and in the walls of the alimentary canal. Select a small vessel and observe carefully its size.

Pith the spinal cord of the frog. After a few moments record the changes which have occurred in the appearance of the heart, the large vessels, the vessels of the alimentary canal, and particularly the small vessel previously selected. How has the destruction of the spinal cord altered the distribution of the blood?

(*Demonstration.*—*The Inhibition of Vasoconstrictor Tone.*—The vasoconstrictor tone can be inhibited by stimulation of the depressor nerve, an afferent nerve from the ventricle and the first part of the aorta.

In the neck of an anaesthetized and tracheotomized rabbit the two small nerves accompanying the vagus are exposed. The nerve which, when stimulated, does not affect the iris is the depressor nerve. A thread is tied tightly about it.

 In one carotid artery a cannula is fastened and connected with a small glass tube about 2 m. high. The tube, the cannula, and the rubber connections are all coated inside with aⁿ layer of pure lubricating oil to delay clotting, or hirudin is injected into a vein, 0.02 gm. per kilo body weight. The clip is now removed from the artery and the blood allowed to rise in the glass tube. To what height does it rise? This was the original method used by the Reverend Stephen Hales to demonstrate the pressure of the blood. When the pressures are in equilibrium the depressor nerve is stimulated. What change occurs in the level of the blood in the tube?

Count the pulsations of the column of blood before and during stimulation. Is there a slowing of the heart rate which would account for the change?

If so, does it occur when the vagus nerve is cut and the stimulation repeated?

Is there any effect on the heart if the electrodes are applied to the nerve peripheral to the ligature?

What effect of stimulation would explain the result observed? (Ludwig u. Cyon, *Ber. d. k. sächs. Gesell. d. Wiss.*, Leipzig, 1866, p. 307.)

Note the effect of rupture of the splanchnic nerves, by pulling on a ligature previously applied.)

Local Vasoconstriction and Vasodilation in Man.—Plunge one hand for a moment into an ice-salt mixture. Withdraw the hand and at once compare its appearance with the other. Is it paler or redder than normal? Explain the appearance.

Plunge the same hand for a moment into water which is as hot as can be borne. Withdraw the hand and again compare it with the normal. Explain the appearance.

Does the appearance of the veins in these two conditions indicate a variation of venous pressure? If so, explain.

The Pressure of Blood in the Arteries

With the factors which control blood pressure determined, the conditions in different parts of the vascular system will next be examined.

Arterial Pressure in the Cat.—In the following observations on the conditions which affect blood pressure in a mammal, the preparations and manipulations are divided among four students:

an operator, an assistant operator, a kymograph operator, and a recorder. Since the procedures involved are unfamiliar, the entire technique will be demonstrated by an instructor on the first day of the experiment. At this time it is essential for each member of the group to become acquainted with the arrangement of apparatus and its use. In all reports of the experiment a diagram illustrating the working of the washout system is required ; it will be of benefit if this is constructed on the day of the demonstration.

On the next day the experiment will be carried through by the students, making use of the experience gained during the demonstration.

On the third day the laboratory period will be devoted to a discussion of the records obtained and the reactions they represent.

In any mammalian blood pressure experiment certain general precautions must be constantly in mind: —

1. Never release an artery until escape of blood into the washout and recording system is made impossible.
2. Wash out after any reaction which carries the blood far into the recording system, because such reactions fill the tubing near the artery with blood undiluted with anticlot solution. After adrenin injections this precaution must be carried out with particular care.
3. Economize drum space. Reactions may be recorded in practical entirety on the drum if control tracings are not too long.
4. Watch the respiration. This will be the best guide if the animal begins to lapse into bad condition.

Anaesthesia. — The cats will have received ethyl carbamate (urethane), 2 gm. per kg. by stomach tube, one hour before operating is undertaken.

Operations. — Make the skin openings *no larger than necessary*. Expose the following structures in the stated order, laying two linen ligatures under each: (1) trachea — 4 cm.; (2) right carotid artery — 3 cm.; (3) right vagus nerve — 3 cm.; (4) left vagus nerve — 3 cm.; (5) left femoral vein — 3 cm.; (6) crural nerve — 3 cm.; (7) femoral artery — 3 cm. Occasionally in the cat the depressor nerve lies as a third strand in the carotid sheath; if present, isolate it and later demonstrate its action.

Tie in cannulae in this order: (1) trachea; (2) right carotid artery; (3) left femoral vein.

Connect the parts of the washout system and drive out the air. With drum smoked and in position, and electromagnetic signal recording five second intervals and adjusted to write a base line for the mercury manometer, preparations for actual recording are now complete.

The Normal Arterial Pressure. — Obtain with the mercury manometer a short record of the normal arterial pressure. Express the mean pressure in figures.

Are there alterations in blood pressure with respiration ?

Are there alterations in heart rate ?

The Effect of Asphyxia. — Fill a small rubber bag with expired air and attach it to the tracheal cannula so that the animal breathes to and fro only the air in the bag. Record the changes with the mercury manometer.

Through what stages do the changes pass ?

What effects are noticeable on respiration ?

What are the changes in pulse rate at different stages of asphyxia ?

The Effect of Sensory Stimulation. — Stimulate the central end of the crural nerve with a moderately strong tetanizing current. Record the pressure at the height of the reaction.

What do you conclude as to the eventual central connections of certain afferent fibers in this nerve?

What is the effect upon the heart rate ?

What are the common initial results upon heart and blood pressure of severe laceration of a peripheral nerve ?

The Effect of Adrenin. — Inject 0.25 c.c. adrenin (1 : 50,000) into the femoral vein. Note the effect on the iris as well as on the blood pressure.

What is the latency of the reaction ?

What is its complete duration ?

Is the pressure following the reaction normal or subnormal ?

If the latter, what dangers may follow the use of adrenin for local hæmostasis ?

How great an increase in systolic pressure is obtained ?

Record the pulse rate during the rise in pressure and at the apex of the rise. Is there evidence of cardiac acceleration, and if so what is its cause ?

Why is the heart slow at the apex of the rise in pressure ?

Why is the adrenin effect transient ?

What was the effect upon the iris ?

Inject 1.0 c.c. adrenin (1 : 50,000) subcutaneously and account for the results.

The Effect of Amyl Nitrite. — Open a bottle containing amyl nitrite and hold its mouth near the tracheal cannula until a change in blood pressure begins. The drug has a peripheral action. Account for the effect observed.

The Effect of Vagus Stimulation. — Cut the right vagus between two tightly tied ligatures. Record with both manometers the effects of stimulating both the central and the peripheral ends.

a. Peripheral Stimulation.

What is the effect on the heart rate ? Does this appear at once ? Is it persistent ?

Record the pressure at the height of the effective stimulation.

Is blood pressure normal or subnormal when the reaction is over ?

b. Central Stimulation.

What is the effect on respiration ?

On heart rate ?

On mean blood pressure ?

The Effect of Bilateral Vagus Section. — Cut the left vagus as the right was cut.

Is there any influence upon respiration not noted on section of the right nerve ?

Stimulate centrally and peripherally and answer again under the new conditions all the questions asked in the foregoing paragraph.

Adrenin Injection after Vagus Section. — Again inject 0.25 c.c. adrenin (1 : 50,000) in the femoral vein. Record the effects.

Account for the difference in the reaction as compared with the first intravenous adrenin injection. Answer by thorough discussion.

Sensory Stimulation after Vagus Section. — Stimulate the central end of the crural nerve.

Account for any difference in effect as compared with previous stimulation.

Consult an instructor before progressing further.

The Effect of Haemorrhage and Saline Injection. — If the mean pressure has fallen to a dangerous level give an intravenous injection of warm 0.85 per cent NaCl solution, recording the effect

on arterial pressure. If the pressure has remained high, bleed twenty-five c.c. from the left femoral artery, and record the effect of this hæmorrhage and a subsequent saline injection.

What are the effects of intravenous saline injection? Is there immediate betterment? If possible attempt to judge of their persistence.

Discuss what in the absence of blood for transfusion you would regard as the most effective solution for intravenous administration in hæmorrhage or critical low blood pressure.

The Effect of introducing Air into a Vein.—Expose the external jugular vein which lies just beneath the skin a few centimetres from the median line. Open the vein and insert a pipette. Blow a small amount of air into the circulation. Observe the result in the curve of arterial pressure.

After the death of the animal open the right auricle. Note the froth in the cardiac cavities.

***The Effect of Gravity on the Circulation.**—Stand with one arm held vertically upward and the other hanging at the side. After a minute compare the appearance of the backs of the two hands as they are brought side by side. The blood has evidently been circulating in the body. Explain the appearance of the hand that was held above the head.

(Demonstration. — Changes of Blood Pressure with Posture; the Nervous Control.—An animal holder is arranged to swing about an axis directly below the point at which a cannula is introduced into the carotid artery. The Hales method may be used or the blood-pressure curve may be recorded. The anaesthetized animal is turned to a vertical head-down position. Is there any change in the blood pressure?

Next the animal is turned to the vertical head-up position. Is there any change?

Is the change recovered from?

The spinal cord is now divided in the lower cervical region. Again the animal is turned to the vertical head-up position. How does the result differ from that observed in the animal with the nervous system intact?

Explain the mechanism whereby the circulation is maintained in the upper parts of the body, when the animal is in the upright position. (See Hill, *J. Physiol.*, 1895, xviii, 15.)

Arterial Pressure in Man.—The observations on arterial pressure already made have shown that there is maintained in the arteries a blood pressure which rhythmically rises with each heart beat and sinks in the intervals; that the highest pressure produced by the heart beat is called the "systolic pressure," the lowest pressure reached before the next beat the "diastolic pressure," and that the difference between the two is the "pulse pressure."

If to the outside of an artery a pressure is applied which prevents the pulse from passing, and this pressure is then lowered until the pulse is just felt beyond the compressed region, the external pressure applied is at this point barely less than the maximum pressure inside the artery. It therefore registers systolic pressure.

If the external pressure exactly balances the diastolic pressure, the artery wall in diastole is neither stretched nor compressed. Under these circumstances the walls are in the state of greatest extensibility, and the excursion of the walls due to the passage of the pulse wave is therefore maximum. If the external pressure is greater or less than the diastolic pressure, the excursions will be less extensive. The degree of external pressure which results in maximum excursions of the compressed arterial wall registers the diastolic pressure of the blood.

An instrument which permits the registering of systolic and diastolic arterial pressure in man is the Erlanger sphygmomanometer. In using this instrument a pressure is applied to the brachial artery by inflating a bag fastened around the arm under a leather cuff. Characteristic changes in the pulsations conveyed to the cuff from the artery, and recorded on a drum, give an objective record of systolic and diastolic pressure (Erlanger, *Am. J. Physiol.*, 1902, vi, p. xxii).

Open completely the valve interposed in the tube leading to the arm cuff and strap the rubber bag to the arm snugly but not so tightly as to obstruct the flow of blood in the veins.

The Method of Palpation. — Without bringing the smoked drum into position determine by palpation the systolic pressure of the subject. To do this, compress the bulb and raise to about 170 mm. the pressure in the arm cuff as measured by the mercury manometer. By releasing the needle valve the pressure in the apparatus gradually falls and the mercury manometer is read as the first pulse is felt in the radial artery. This reading represents the systolic pressure. Make several check determinations.

Why do these readings represent the systolic pressure ?

The Method of Auscultation. — With the bell of the stethoscope in the antecubital space just below the cuff repeat the foregoing observations. Record the level of the mercury column when

- (1) The sound first occurs,
- (2) The sound changes, and
- (3) The sound entirely disappears.

Make several check determinations.

The Method of Graphic Record. — Place the smoked drum in position for recording and introduce an electromagnetic signal below the sphygmomanometer lever. Raise the pressure in the cuff to a point 20 mm. above the systolic pressure as recorded by the auscultatory method. Set the writing lever against the drum and make a series of records each covering at least two respirations, allowing the pressure to drop 10 mm. after each record while the drum is stopped. Continue recording until the excursions of the lever have passed their maximum.

Why does the maximum excursion of the lever record the diastolic pressure ?

Repeat the procedure given above but combine the auscultatory method with it and record by means of the electromagnetic signal, when you hear

- (1) The first sound,
- (2) The change of sound, and
- (3) The last sound.

Repeat these observations allowing the pressure to fall in steps of 5 mm. instead of 10.

The Effect of Vigorous Exercise. — Disconnect the arm cuff from the rest of the apparatus but leave it on the arm ready for use. Test the effect of such exercise as running several times up and down stairs. Attain some measure of breathlessness in this exercise.

Tabulate your results for all methods, reporting systolic pressure, diastolic pressure, pulse pressure, and mean arterial pressure.

The Effect of Respiration. — Adjust a pneumograph to the subject, connect it with a writing lever, and adjust the three writing points (for blood pressure, respiration, and signal) so that they write in approximately the same straight line. Before starting the drum make simultaneous ordinates from which future measurements may be made.

Bring the pressure in the arm cuff to the point of determined mean arterial pressure in the individual. Start the drum and make records, breathing normally, deeply and slowly, and deeply and fast.

Does the heart rate change with any phase of respiration ?

Adjust the pressure in the cuff to the systolic level. Breathe deeply and slowly and record the sounds by means of the stethoscope and electro-magnetic signal.

Can you determine a pressure level at which the sounds are heard only with inspiration or only with expiration ?

If so, what do you infer as to the regular effect of respiration on the systolic pressure of the individual examined ?

With the three levers in place and the subject breathing at the rate of about five pulse beats to the inspiratory phase of respiration make a record of the sounds from their incidence to their disappearance.

Do the sounds appear during inspiration or expiration ?

Where do they disappear ?

Submit your records to the instructor and go over with him the various criteria by which systolic and diastolic pressures are estimated.

The Pulse

The rhythmic discharge from the heart into the arteries produces, as has been seen, rhythmic oscillations of pressure—the pulse pressure. Thus the aorta and other arteries near the heart are expanded and receive the extra amount of blood. The pressure causing the expansion is transmitted progressively to more and more remote arteries, causing them in turn to expand and receive the blood forced out of the aorta. When the semilunar valves close, the arterial walls recoil elastically from the stretching, and force the extra blood onward into the capillaries. First the aorta sinks back to its diastolic size, then progressively the arteries leading off from the aorta, and finally the arterioles. A fresh discharge from the heart causes a new wave of expansion and contraction to sweep through the arterial system. At any point the rise and fall of the vessel wall can be felt as the wave passes. This is “feeling the pulse.” Its importance lies in the easy method it offers for finding the rate of heart beat, and for judging roughly the state of the circulation in the arterial system.

The Feeling of the Pulse.—Place the tips of the first three fingers of one hand over the radial artery of the opposite wrist. Carefully observe the pulse. Four characteristics are noted.

(a) *Rhythm.* Is the pulse rhythmic ?

What is the rate per minute ?

How accurately can this rate be judged by counting during 15 seconds ?

How is the rate affected by standing, sitting, reclining, and by exercise ?

(b) *Tension.* Compress the artery with the finger nearest the heart until no pulse is felt by the other fingers. The amount of compression has obliterated the artery. Thus the systolic pressure can be roughly judged. A “soft” or “low tension” pulse is easily obliterated, a “hard” or “high tension” pulse requires considerable pressure to stop it.

(c) *Form.* Does the pulse rise to its maximum rapidly or slowly ? If rapidly, it is “quick”; otherwise, “slow.” These terms apply to the systolic period only and do not apply to the rhythm.

There is a characteristic second wave in the pulse curve, usually slight, but occasionally so marked as to be felt separately. This "dicrotic" wave is especially observable in conditions of low tension in the arteries.

(d) *Amplitude*. The degree of excursion of the arterial wall depends on several factors; the output from the ventricle, the quickness of the output, the tension in the arteries, and the elasticity of the arteries.

Study these four characteristics — *a*, *b*, *c*, and *d* — repeatedly under different conditions and on different persons, and thereby establish an experience with the normal pulse and its variations.

The Movement of the Pulse Wave. — With the tips of the first fingers of the right hand feel the pulse in the left carotid artery. While the carotid pulse is thus being felt, apply the fingers of the left hand to the radial artery in the right wrist. Both the carotid and the radial pulsations are now felt. Which is earlier? Why?

Inhibition of the Vagus Impulses. — The cutting of the vagus nerves, as already seen, results in a more rapid heart beat. The vagus nerves therefore normally exercise a continuous restraining influence on the heart rate. This influence can be inhibited in the central nervous system.

Fill a small beaker with water. Count the heart rate, feeling the pulse at the wrist. Sip the water slowly, and while sipping, let the heart rate be counted again. What has been the effect of the swallowing?

Arterial and Venous Pulse Records in Man; Cardiac Impulse — With the subject prone upon a table and with collar loosened make records of the pulsations of the carotid artery. In using the delicate tambour provided it is essential to find a point from which pure carotid pulsation is obtained, free from any jugular element. When such a spot has been found and marked, secure a record of jugular pulse with the second tambour. Holding the breath will facilitate the securing of this record. This jugular pulse record is, however, of very slight value by itself.

Set the two levers in line and make simultaneous ordinates. Adjust the receivers so that one tambour records the jugular and the other the carotid pulse.

Make a record of the cardiac impulse, using a funnel for a receiver and, if the impulse is very large, a writing tambour such as has been provided for recording respiration.

Secure synchronous records of jugular pulse and cardiac impulse, and carotid pulse and cardiac impulse.

Note the form of the radial and carotid pulses.

Measure out the A, C, and V waves of the jugular pulse.

Why is a jugular pulse tracing alone of little value?

What event does each of its waves record?

What is the significance of the A-C interval?

Is it of normal length in the individual examined?

An absent A wave would indicate what?

How would you determine the absence of this wave?

Has the cardiac impulse as obtained in the members of your group any characteristic form?

The Flow and Pressure of Blood in the Veins

The following demonstration shows how greatly the pressure given the blood by the contraction of the heart has been exhausted by the time the blood is approaching the heart in the veins. With this low pressure other agents than the heart beat become prominent as accessories to the circulation.

(Demonstration.—A Comparison of Arterial and Venous Pressure.—A cat is etherized and the carotid pressure is measured by the method used by Stephen Hales. Before removing the clip from the artery the cannula and the glass tube are filled to a height of 1.5 m. with sodium carbonate solution (specific gravity 1.08) colored with methylene blue. A glass tube, bent at a right angle and rising about 15 cm., is introduced towards the heart in the innominate vein and tied in place. The tube should be filled with the sodium carbonate and methylene blue solutions, and these solutions should rise about 8 or 9 cm. in the tube, when clamped in a vertical position.

Remove the clip from the innominate vein and push the tube downward about 3 cm. into the superior vena cava. Remove the clip from the carotid artery.

Compare the heights of the two columns. Compare the cardiac and respiratory pulsations. Explain the pulsations in venous pressure. Compress the abdomen and note the change in both pressures. Compress the thorax and hinder the filling of the heart; how are the two pressures affected ?)

The Valves in the Veins.—Expose the right forearm. Swing the arm or compress the vessels near the elbow until the veins of the forearm stand out prominently. Lay the index finger of the left hand on one of the veins near the wrist, and with the thumb press the blood in the vein from the finger towards the heart. If the blood flows back to the finger, lay the finger at the point the thumb reached and repeat the experiment. If necessary, continue this procedure. A point will be found beyond which the blood will not return towards the compressing finger. This point marks the position of a valve. With the finger laid just below the valve, attempt by pushing downward over the vessel with the thumb to force the blood back past the valve. Is the valve competent? Find other valves in the veins of the forearm and the back of the hand (Harvey, *The Motion of the Heart and Blood Vessels*, 1628).

The Effect of Muscular Contraction.—Compress the veins near the elbow and note the length of time required for the veins to become prominent. Pass the hands over the surface from wrist to elbow and empty the veins. Again compress the veins near the elbow, but now rhythmically and forcibly close and open the hand. Compare the periods required to fill the veins during muscular quiet and during muscular contraction. Consider the relations of external pressure on a vein to the action of the valves, and explain the effect of muscular contraction just observed. Cite occupations in which muscular activity affects the circulation.

Venous Pressure in Man.—A rough indication of venous pressure may be obtained by observing the dilated veins on the back of the hand as the hand is gradually and slowly raised. Note how high above the right auricle the hand is when the veins collapse.

What is the effect on venous pressure of taking a deep breath and then contracting the expiratory muscles while the glottis is closed? Explain.

(*Demonstration. — Circulation Time.* — In a rabbit anaesthetized with urethane the carotid artery is exposed, and under it is placed a strip of white glazed paper resting on a piece of thin sheet rubber. A strong illumination is thrown on the artery. Into the jugular vein of the opposite side is introduced a cannula pointed towards the heart. To the cannula a burette is attached, filled with a saturated solution of methylene blue in isotonic salt solution at body temperature.

The cannula and connections are also completely filled with this fluid. The burette is held upright with the fluid at a high level. Now the clip is removed from the vein and, at a signal from an observer with a stop-watch, the burette is opened and 0.5 c.c. of the contents permitted to enter the vein. It should run in rapidly. The observer stops the watch when the blue appears in the artery. As the methylene blue is quickly reduced to a colorless compound, this observation can be repeated several times. The same amount should be injected each time. What is the average of the observations? (Hering, *Ztschr. f. Physiol.*, 1829, iii, 85; 1833, v, 38.)

The time thus determined is approximately that required for the passage of the blood through the pulmonary circulation.)

SECRETION

AMONG the functions performed by the blood and lymph is that of providing the materials for secretion. These materials pass into gland cells from vascular and perivascular spaces and, after being more or less modified in the cells, are transmitted to the channels leading away from the gland. The secretion of the cells is dependent on a supply of blood and lymph, indeed the cells may be activated by special stimulating substances brought by the circulating fluids. There is evidence, however, that the secretion is also controlled by nervous impulses affecting the secreting cells. The chief phenomena of secretion can be observed in a study of the most important digestive glands.¹

***Saliva.**—Saliva is normally secreted in abundance when sapid food is being chewed. It may flow abundantly also when merely appetizing food is seen or remembered. It will flow also when irritating substances are introduced into the mouth, acting in these instances as a diluent to protect the mucous membrane.

After rinsing the mouth with water, inhale through the mouth the vapor of acetic acid or hold in the mouth for a moment a 0.5 per cent solution of acetic acid, or a few drops of ether. What is the effect on the secretion of saliva?

What is the effect of saliva on the action of the irritating substances?

(Demonstration.—The Influence of Nerves on the Secretion of the Submaxillary Gland.—A cat is anaesthetized, tracheotomized, and maintained in a state of anaesthesia until death. Between the front teeth is inserted a small stick. A cord is tied around the jaw immediately behind the stick, and the ends of the cord fastened about an upright on the animal holder so that the under side of the lower jaw is fully exposed.

A median incision is now made through the skin from the tip of the jaw to the base of the neck, and with a weight the flap is pulled aside. A large transverse vein is revealed; it is tied at two points and cut between them. The digastric muscle is observed lying along the inner side of the ramus of the jaw; a ligature is tied about it at the posterior end of the visible portion and the muscle removed anterior to the ligature. The mylo-hyoid muscle—a thin sheet with fibres extending inwards from ramus to median line—is now revealed. The muscle is cut along the middle line, laid back to the jaw, and excised. Now the submaxillary gland is exposed so that it is readily observed. (See Reighard and Jennings, *The Anatomy of the Cat*, New York, 1901, p. 109.)

With the removal of the mylo-hyoid muscle the lingual nerve can be seen passing inward from under the jaw and forward with two small ducts—the ducts of the sublingual and submaxillary glands. A fine white strand passing backward from the junction of the lingual with the ducts is the chorda tympani nerve. A ligature is tied about the combined

¹ With proper expert assistance the demonstrations in this chapter may be made class exercises.

trunk of the lingual and chorda tympani as far under the jaw as possible and the trunk severed central to the ligature.

By means of the ligature the nerves are lifted and stimulated with a weak interrupted current. The duct of the submaxillary gland—the inner duct—fills with saliva and becomes prominent. With a blunt dissector the duct is isolated for about a centimeter, a silk thread fastened around it as far forward as possible, and another silk thread arranged to tie about a cannula. When the duct is full, a bit of it is lifted gently in fine forceps, and with small scissors a V-shaped cut is made. Into the opening a very small cannula is introduced and tied in place. To the cannula is attached a glass tube of small bore, supported at a slight incline towards the duct.

Now the chorda tympani nerve is stimulated with a weak interrupted current. Does the appearance of the gland change?

Saliva will flow into the tube. Is there a latent period?

Does the flow persist after the cessation of the stimulus?

If repeated demonstration of the effect is desired, a tube may be introduced between the cannula and the glass tube, and from time to time the saliva may be allowed to flow out of the T-tube until the meniscus is again brought to the lower end of the incline.

The cervical sympathetic trunk on the operated side is ligated and severed. By means of the ligature the cephalic end of the nerve is lifted and stimulated. Is the saliva flow the same as after equal stimulation of the chorda fibres? (Ludwig, *Ztschr. f. rat. Med.*, 1851, n. F. i, 259; Bernard, *Gaz. méd. de Paris*, 1857, p. 696.)

Compare the physical consistency of the saliva resulting from the two stimulations. The statements on this point found in most text-books are based on observations on the dog.

The cannula is next connected with a mercury manometer and the chorda tympani stimulated for some time, with occasional periods of rest. Note the pressure which the process of secretion may occasion. Compare the pressure with blood pressure.

If time permits, the following effects of drugs may also be demonstrated.

Ten mgms. of nicotine in a 1 per cent solution are injected intravenously. What now is the effect when the chorda and sympathetic are stimulated?

When the nicotine effect has passed off (about 15 minutes), 10 mgms. of atropine sulphate are injected. What now is the effect of stimulating the chorda and sympathetic?

Into the gland through the cannula and the duct a small amount of 2 per cent solution of pilocarpine is introduced. Is the normal condition now restored?

Gastric Juice. **(Demonstration. — Psychic Secretion of Gastric Juice. Bidder and Schmidt's Observation. —* A cat is not fed for 36 or 48 hours, but is supplied water. A plate of fish is now placed outside the cage for about a half hour. At the end of that time, the animal is quickly etherized to death. Ligatures are tied tightly about the cardiac and pyloric sphincters and the stomach removed. The stomach is opened over a clean glass dish, and the contents tested for gastric juice—by the acid reaction and the ability to digest fibrin. (Bidder u. Schmidt, *Die Verdauungssäfte und der Stoffwechsel*, Leipzig, 1852, p. 35.)

Pancreatic Juice and Bile. *(Demonstration. — The Action of Secretin on the Flow of Pancreatic Juice and Bile. —* From one of the cats used for previous demonstrations the first two feet of the small intestines are removed and slit lengthwise, and the mucosa washed in running tap water. The gut is now laid on a dry wooden support with the mucous side exposed. With a dull knife the mucous membrane is gently scraped away, rubbed well with sand

in a mortar, and may then be allowed to stand for a brief period under two or three times its volume of 0.4 per cent hydrochloric acid.

Shortly before it is to be used, the mixture is boiled in a porcelain dish, and while boiling made alkaline with strong caustic soda. Then it is made slightly acid with acetic acid. The mixture is now strained and pressed through muslin, and the fluid portion filtered through paper. The filtrate contains secretin.

A cat is etherized and maintained in anaesthesia until death. The femoral vein is exposed and a cannula introduced pointing towards the heart. After an incision along the mid-ventral line into the anterior part of the abdominal cavity, the duodenum is found and withdrawn. On the right side of the gut, as withdrawn, a small projection of fat reaching from the pancreas on to the intestine is noted. If the wall of the gut is pulled tightly over a finger-tip placed beneath this region, a broad white line is observed reaching inward from the gland. This line, usually closely accompanied by a blood vessel, is the main pancreatic duct. The bile duct runs nearby, but anterior, in the wall of the intestine; be careful to avoid it.

By means of a curved, small, blunt dissector a silk ligature is carried through the tissues around the duct. With a sharp, thin-bladed scalpel a cut is made directly downwards through the intestinal wall, transverse to the duct near its termination, and avoiding, if possible, the accompanying blood vessel. The end of the severed duct is seen in the cut. Into it is introduced first a tapering seeker to stretch slightly the opening, and then a glass cannula. The cannula is tied in place by means of the silk ligature. In the entero-hepatic omentum the common bile duct is found and into that another cannula is fastened. The cystic duct should be tied.

The animal is now turned so that the bile and pancreatic juice will each drop on a nearly balanced indicator (such as was used in showing the rate of lymph flow from the thoracic duct) before falling into a receptacle.

After the rate at which these secretions drop from the ends of the cannulae has been determined, a small amount of the preparation of secretin is injected through the cannula in the femoral vein. Let some members of the class count the oscillations of the indicator showing the flow of the pancreatic juice, while others observe that registering the flow of bile.

Does the secretin cause an increased flow of the pancreatic juice and bile?

If time permits, after repeated demonstration of the action of secretin, the cat is atropinized, and again secretin is introduced. Is the action of secretin affected by the atropine?

Instead of injecting prepared secretin into a vein, 0.4 per cent HCl may be injected into an isolated loop of jejunum. The mucosa of the injected loop will then furnish secretin to the body. (Bayliss and Starling, *J. Physiol.*, 1902, xxviii, 325.)

Test the pancreatic juice at body temperature for its amylolytic power with thin starch paste colored with iodine; for its lipolytic power with neutral olive oil and a dilute sodium carbonate solution (0.25) per cent; and for its proteolytic power with strands of fibrin. It will be found that pure pancreatic juice as secreted is incapable of attacking the protein. The trypsinogen must first be activated.)

(*Demonstration.* — *The Activation of Trypsinogen by Enterokinase.* — From one of the cats used for a previous demonstration the upper part of the small intestine is removed and the

mucous membrane gently scraped away as was done in preparing secretin. The scrapings are allowed to macerate two days in water in a closed vessel, containing a few drops of chloroform to prevent bacterial decomposition. Just before being used the macerated mucosa is filtered through filter paper.

In fresh pancreatic juice some strands of fibrin are placed, and then are added a few drops of the filtrate resulting from the maceration of intestinal mucosa. The mixture is kept at body temperature. Compare the result with that which followed placing the fibrin in pure pancreatic juice. (Schepowalnikow, see Pawlow, *The Work of the Digestive Glands*, Engl. trans., London, 1902, p. 159.)

DIGESTION

THE introduction of food into the body results from the combined action of two classes of factors, — physical or mechanical, and chemical. The mechanical factors triturate the food, transmit the food into regions where the digestive juices are secreted, mix the food with these secretions, expose the digested food to the absorbing mucous surfaces, and rid the body of the useless waste.

The Mechanical Factors

***The Effects of Comminution on Amylolytic and Proteolytic Digestion.** — By chewing rubber or paraffine secure half a test-tube of saliva. Filter it and pour equal parts into two test-tubes. To one add a small cube of starch jelly stained with carmine, to the other add the same amount of starch jelly after dividing it as finely as possible.

Let digestion take place at 40°C. in the two test-tubes for the same length of time. From the color of the solution determine in which test-tube digestion has proceeded more rapidly. What conclusion may be drawn?

Repeat the experiment with cubes of coagulated egg albumin stained with carmine, and placed for digestion in artificial gastric juice. The artificial gastric juice may be made from 0.4 per cent hydrochloric acid and commercial pepsin. (See Grützner, *Arch. f. d. ges. Physiol.*, 1874, viii, 452; 1876, xii, 293.)

***The Lubricating Action of Saliva.** — With a clean cloth wipe dry the interior of the mouth and observe with how much friction the tongue and cheeks are moved in the absence of saliva.

While the mouth is dry bite off a large part of a dry cracker and endeavor to masticate and swallow it. The function of lubricating the food for ready swallowing can now be appreciated.

***The "Pill Reflex."** — Attempt to swallow an empty gelatine capsule. Usually there is difficulty in forcing an unchewed solid object into the pharynx. There is a spasmodic action of the muscles at the back of the mouth and the object is pushed forward again to the front of the mouth. This reflex can be so developed that no solid food is easily swallowed.

***The Mechanism of Deglutition.** — Take some water into the mouth. Note carefully how the tongue, pressing against the hard palate in front and at the sides, closes the exit forwards. Swallow the water. Observe that the tongue is suddenly pressed against the hard palate from before backwards, while the soft palate rises and shuts off the exit into the nose. The water is forced into the only outlet, the pharynx and œsophagus.

***Deglutition Sounds.** — The sudden rise of the tongue is due to a quick contraction of the mylo-hyoid muscles. A sudden rise of pressure is thus produced in the mouth and any liquid contents are shot along the œsophagus at a rapid rate.

In a quiet room listen with a stethoscope placed over the end of the sternum, or over the sixth and seventh ribs immediately at the left of the vertebral column. While thus listening let the subject take a swallow of warm water. How many sounds are heard? Note the time interval after the swallowing (Meltzer, Cbl. f. d. med. Wiss., 1883, p. 1).

(*Demonstration.*—*The Innervation of Deglutition.*—A rabbit is anaesthetized with ether. The trachea is carefully dissected from the œsophagus, without injury to the nerves lying between the two structures. A silk thread is looped beneath each vagus nerve. At the level of the lower part of the thyroid cartilage the vagus nerve is joined by the superior laryngeal: a thread is passed beneath this nerve.

The trachea is severed as low in the neck as possible, and into it is tied a tracheal cannula. The piece of trachea between the cannula and the cricoid cartilage is now removed, thus revealing the œsophagus.

The stomach is exposed and opened. Into the œsophagus through the cardia is fastened an upright L-shaped glass tube containing water.

The superior laryngeal nerve is now stimulated with a weak interrupted current until the rabbit swallows. When the swallowing movement is made, watch for the peristaltic wave to pass along the cervical œsophagus, and observe the rising of the water in the tube.

Cut the right vagus nerve low in the neck and again test for the presence of a peristaltic wave along the œsophagus.

Cut the left vagus nerve and repeat the test.

What conclusion may be drawn as to the nervous mechanism of swallowing and the part played by the vagus nerves? (See Reid, *Edinburgh M. and S. J.*, 1839, li, 274; Mosso, *Unters. z. Naturl. d. Mensch. u. d. Thiere*, 1876, xi, 331.)

Stomach and Intestines. (*Demonstration.*—*Peristalsis of the Stomach, Rhythmic Segmentation in the Small Intestine, Antiperistalsis in the Large Intestine.*—A tank, large enough to receive the extended body of a cat, is filled with isotonic salt solution (mammalian), warmed to body temperature, and maintained at that temperature during the following experiment.

About one hour before the experiment a cat, which has been fed the previous afternoon, is given 100 c.c. of bread and milk to which has been added meat extract. At the end of the hour the cat is etherized and maintained in anaesthesia until death. A small opening is made between the laminae of contiguous vertebrae in the lower lumbar region, a flexible wire introduced, and the spinal cord destroyed through the thoracic region. The wire is at once withdrawn, and the small opening in the skin is closed with sutures.

A longitudinal incision is now made through the skin over the linea alba and any slight bleeding is checked. The animal is fastened back downward in the tank, with care that the nose and mouth are safely above the surface of the salt solution.

The abdominal cavity is opened along the linea alba and the free edges fastened widely separated. After the omentum is lifted, the stomach and small intestine are clearly revealed. (See v. Braam Houckgeest, *Arch. f. d. ges. Physiol.*, 1872, vi, 267.)

The peristalsis of the stomach is first observed. Over what part of the stomach do the waves pass?

Does each wave force food through the pylorus?

What must be the effect of the waves if they do not propel the food?

The process of rhythmic segmentation in the small intestine is noted. What is the significance of the local change of color of the canal where the rings of constriction occur?

If antiperistalsis does not appear in the large intestine, introduce a thin mush into the colon from the ileum. Observe the frequency of the waves. What must be the effect of antiperistaltic waves pressing the food into a blind pouch?

What is the importance of the ileo-colic sphincter? (See Cannon, *Am. J. Physiol.*, 1902, vi, 251.)

If there is delay in the appearance of these activities they can be started by attaching to the tracheal cannula a rubber tube, about 30 cm. long; or by painting on the cardiac end of the stomach and on the colon a 5 per cent solution of barium chloride, which will cause rings of tonic constriction to develop and later to pulsate and send off waves.)

(*Demonstration.*—*The Two Parts of the Stomach.*—Upon completing the foregoing demonstration the animal is killed. Ligatures are tied tightly around the pylorus and the cardia, and the stomach removed. The organ is laid on a flat surface and opened lengthwise.

Note the difference in the consistency of the food in the pyloric and cardiac portions. (Eberle, *Physiologie der Verdauung*, Würzburg, 1834, p. 100.) Apply test papers to the pyloric contents, to the surface of the mass in the cardiac portion, and to the interior of this mass. Relate the results to the observations already made on gastric peristalsis.)

The Nervous Control of the Alimentary Canal

Food is carried through the alimentary canal and is churned in the stomach and large intestine by means of peristaltic waves. Peristalsis as seen in the cervical œsophagus was dependent on the integrity of the vagus supply. In that part of the œsophagus the canal is provided only with striped muscle. In the lower œsophagus of many animals and throughout the remainder of the alimentary tract, the musculature is non-striate in character, and is provided in the myenteric plexus with an intrinsic nervous regulation. The primitive nerve net between the two layers of the muscularis is the seat of a local reflex, such that a stimulus at any point causes a contraction above and a relaxation below the stimulated point. It is thus that peristalsis is regulated.

***The Myenteric Reflex.**—Support an L-shaped glass rod in a beaker containing Ringer's solution at body temperature. Arrange a heart lever to write on a smoked drum. When this apparatus is ready for use remove from a pithed cat a piece of small intestine about 3 cm. in length. Or the piece may be severed from a large isolated extent of the gut kept alive in oxygenated Ringer's solution at body temperature. Leave a small tab of mesentery attached to the rectal end. By means of a needle pass a loop of silk thread through the serosa and muscularis of the short piece at a point midway between the two ends. Tie the thread loosely. At an opposite point insert another loop of the thread. Fasten the latter thread to the L-shaped glass rod, so that the rod holds the intestine in the warm Ringer's solution. Attach the other thread to the heart lever. As the intestine rapidly dies unless provided with oxygen, it is necessary to make the following observations as soon as possible after the removal of the intestine from the body, or from the oxygenated Ringer's solution.

With small forceps gently pinch the piece of gut about 0.5 cm. below the attachment of the threads, *i. e.*, on the rectal side of the recording ring of muscle. What is the effect of stimulating below the recording ring?

When the normal state of the muscle is resumed, pinch above the recording ring with as nearly as possible the same stimulus as was used before. What is the effect of stimulating above the recording ring? (Bayliss and Starling, *J. Physiol.*, 1899, xxiv, 99; Magnus, *Arch. f. d. ges. Physiol.*, 1904, cii, 132.)

***The Influence of Tonus on Peristalsis.** — Tie a ligature around the pylorus of a frog, fill the stomach with isotonic salt solution, and after tying another ligature at the cardiac end, remove the stomach from the body. Hang the stomach with the cardiac end dipping in a solution of 0.6 per cent sodium chloride and 0.1 per cent sodium carbonate. The portion in this solution should exhibit a tonic contraction, and from this ring of greater tonus peristaltic waves should pass over the rest of the stomach.

When several such waves have passed, wash the stomach in isotonic salt solution and hang it with the pyloric end dipping in the solution containing sodium carbonate. Is the direction of the peristaltic waves reversed?

(*Demonstration.* — *The Extrinsic Innervation of the Stomach and Small Intestine.* — A cat is etherized and anaesthesia continued until death. One of the vagi in the neck is prepared for stimulation. The abdominal cavity is opened along the linea alba, and the free edges of the abdominal wall supported so that the alimentary canal is clearly seen. The left splanchnic nerve is found and cut. Covered electrodes are applied to the peripheral portion of the cut nerve. Throughout the following observations the exposed surfaces of the canal are kept moist with warm Ringer's solution.

The vagus nerve is stimulated with a weak interrupted current. What is the effect on gastric peristalsis and the movements of the coils of intestine?

While the stomach is contracting, the splanchnic nerve is stimulated. Note its effect. Note also the effect of splanchnic stimulation on the movements of the small intestine.

What conclusion may be drawn as to the actions of the vagus and splanchnic nerves on the movements of the alimentary canal?)

***The Chemistry of Digestion**

Feed cats that have been deprived of food for 36 hours and that have received two teaspoonfuls of castor oil about 12 hours before feeding, a mixture of crackers (containing no sugar), neutral olive oil, and boiled lean beef. About 50 c.c. of the mixture may be fed to each animal.

At the end of two hours rapidly chloroform the animals. Let each group of students then make the following observations:

Tie one ligature tightly about the cardia, two about the pylorus, and one about a point approximately three feet along the small intestine. Cut between the two ligatures at the pylorus. After removing the stomach and intestine and washing off the outside, open each in a clean dish.

By methods learned in the course in biological chemistry test the original food for sugar, for fatty acids, and for proteoses and peptones.

Make the same tests on the gastric contents.

Make the same tests on the contents of the small intestine.

Draw conclusions as to the regions in the cat in which carbohydrates, fats, and proteins are first split by the digestive juices.

*ABSORPTION

THE digested food is absorbed chiefly in the small intestine. In the small intestine the segmenting movements, by repeatedly exposing the digested food to the mucosa, must distinctly favor the absorptive process, but these muscular contractions are not essential to the passage of food through the absorbing wall. The essential factor is the activity of cells in the mucous membrane. These cells act differently, however, in different parts of the alimentary canal.

Absorption of Water from the Stomach and from the Small Intestine.—Pith the brain of a tortoise which has not eaten for several days. Tie ligatures about the cardiac and pyloric sphincters, and fill the empty stomach with a measured amount of water. Similarly tie off the small intestine and introduce into it a measured amount of water.

After two hours measure the water in the stomach and in the small intestine.

What conclusion may be drawn as to the absorption of water in these two portions of the alimentary canal? (See Edkins, J. Physiol., 1892, xiii, 454.)

Absorption of the Various Food Stuffs from the Small Intestine

In observations on the absorption of various food stuffs from the small intestine, cats anaesthetized with urethane are employed. The animals are deprived of food about thirty-six hours, and receive two teaspoonfuls of castor oil about twelve hours before the experiment. The operations are done with the help of an instructor. For each injection a loop of small intestine about 40 cm. in length is isolated by ligatures. Several such loops can be prepared in one animal and thus the observations may be increased in number. After the injections have been made, close the abdominal wall with sutures.

Carbohydrate.—*Absorption of Dextrose compared with Absorption of Sodium Sulphate.*—Introduce into one clean loop of the small intestine 20 c.c. or more of 1.0 per cent dextrose solution, and into another clean loop of equal length the same amount of 1.0 per cent solution of sodium sulphate.

After an hour chloroform the animal, measure the liquid content of the two loops, and determine the amount of dextrose and sodium sulphate still not absorbed.¹ Express the results in terms of the percentage of absorption of water and of dissolved substance.

¹ In determining the amount of dextrose use the method already learned in the course in biological chemistry.

In determining the amount of sodium sulphate, first filter the intestinal contents and wash the residue. Boil the filtrate, acidulate with a few drops of hydrochloric acid, and add a hot solution of barium chloride until barium sulphate ceases to be precipitated. Boil for a few minutes, then wait for the precipitate to settle. Pour off the clear liquid through a filter with ash of known weight. Repeatedly boil with water the precipitate in a beaker. Pour the precipitate on the filter. Wash with boiling water, dry, and heat to redness in a weighed crucible. When cold weigh again. Knowing the atomic weight of barium sulphate and the weight of the ash of the filter, calculate the amount of sodium sulphate not absorbed.

Compare the absorption of dextrose and sodium sulphate. Sodium sulphate is more diffusible than dextrose. Do substances pass through the alimentary canal by physical diffusion? (Rohmann, Arch. f. d. ges. Physiol., 1887, xli, 457.)

Protein. — *The Absorption of the Products of Pancreatic Digestion of Protein.* — Pig's pancreas, freed from fat and finely divided is covered with water, and about 5 c.c. of chloroform added. The mixture is placed in a tightly corked bottle and set aside in a warm room for a month or more.

Strain the mixture through cotton to remove the large particles of undigested matter, and then filter through filter paper. The filtrate should not give the biuret reaction. By use of methods learned in the course in biological chemistry determine the total nitrogen in a sample of the filtrate. Make a solution containing 1 g. of nitrogen, and introduce it into a clean loop of intestine. After two hours remove the loop, wash it, slit it lengthwise, wash out the contents, filter them, and press the filter. Determine the total nitrogen in the filtrate. How much has disappeared?

Absorption not a Process of Simple Osmosis or Filtration. — Obtain 50 c.c. of mammalian serum by defibrinating and centrifugalizing the blood.

Select two clean loops of the small intestine equal in length and tie them at either end so that they can be distinguished. Wash out one loop with isotonic salt solution, the other with distilled water or isotonic salt solution plus 0.1 per cent sodic fluoride, which will destroy the cells of the mucosa. Introduce without pressure equal amounts of the serum in the two loops.

After one hour remove the loops and measure the quantity of the contents. The absence of pressure when the serum was introduced eliminated the factor of filtration. (Absorption occurs when the intra-intestinal pressure is less than the pressure in the mesenteric veins.) How is the factor of osmosis excluded?

What does the differential disappearance of the serum in the two loops indicate? (Reid, J. Physiol., 1898, xxii, p. lvi.)

Fat. — *Absorbed Fat in the Villi and Lacteals.* — Feed a hungry cat milk with cream added. After two hours chloroform the cat and expose the mesentery. Note the lacteals and mesenteric lymph nodes. Expose the thoracic duct in the thorax, open it, and note the chylous flow.

Remove some of the upper part of the small intestine, wash it, cut it into small pieces, fix it in a 1 per cent solution of osmic acid, and make a teased preparation of the cells of the mucosa in dilute glycerine. Examine these preparations with a microscope. Osmic acid stains black the droplets of fat and fatty acid. Observe the droplets inside the cells.

This experiment may be varied by feeding the cat butter stained with Sudan III. Two hours after the feeding, the animal is chloroformed. Examine the lacteals. Is chyle present? If so, is it stained or white?

Press some teased mucosa under a cover-slip and examine it with a microscope. Are there stained fat drops in the cells?

If not, apply to the pressed tissue Sudan III in 80 per cent alcohol, wash off the excess, and again examine under the microscope. Are stained fat drops now seen?

Assimilation Limit. Alimentary Glycosuria. — If too much albumin or too much sugar is present in the small intestine at any one time, the absorption may be so rapid that the

alteration of the albumin in the intestinal wall or the storage of sugar in the liver may not be equally rapid. In that case the albumin or the sugar appears in the urine. (Hofmeister, Arch. f. exp. Pathol. u. Pharmakol., 1889, xxv, 240; 1890, xxvi, 355.)

Collect in a metabolism cage the urine of a cat, and examine it for sugar. If none is found, give the cat by stomach tube 50 c.c. of strong glucose solution. Test for sugar the next urine that is passed.

Speed of Absorption and Secretion. — In 10 test-tubes place a small amount of a mixture of thin starch paste (5 parts) and concentrated nitric acid (2 parts). Let one of each pair of students, who has had a small breakfast, swallow a carefully wiped gelatine capsule containing 10 grains of potassium iodide. Let the subject at once wash out his mouth and, by adding some of the wash water to the contents of one of the test-tubes, make sure that it contains no potassium iodide. By chewing a piece of rubber increase the flow of saliva. At two-minute intervals, after swallowing the capsule, expectorate the accumulated saliva into one of the test-tubes and mark on the tube the time since the swallowing. Rinse the mouth with water and begin a new collection.

How much time is required for the potassium iodide to appear in the saliva?

Absorption from the Large Intestine

The large intestine, especially the first portion, is the region where normally water, which has aided the chemical and mechanical changes of the food in the stomach and small intestine, is largely removed from the waste. In this region also the gases resulting from putrefaction may be absorbed. The amount of absorption of nutriment in the colon is slight (Macfadyen, Nencki, u. Sieber, Arch. f. exp. Pathol. u. Pharmakol., 1891, xxviii, 311).

(Demonstration. — Absorption of a Gas by the Large Intestine. Rectal Anaesthesia. — A small bottle is half filled with ether, and fitted with a cork perforated by a glass tube. To the glass tube is attached a short length of rubber tubing about 7 mm. in diameter and provided with side openings near the free end. This end is smeared with softened soap, and introduced into the rectum of a rabbit. The ether bottle is then set in water at 40° — 45° (ether boils at about 35°).

Note when the abdominal and other reflexes disappear. How many minutes have elapsed since the ether began to boil? Smell the breath of the rabbit. What element of safety does rectal anaesthesia possess over anaesthesia by inhalation? Where may unpleasant odors of the breath originate? (See Meltzer, Am. J. Physiol., 1898, i, p. viii; Cunningham and Lahey, Boston M. and S. J., 1905, clii, 450.)

THE BODY FLUIDS AND THEIR REGULATION

As already mentioned, the body is composed of about sixty-five per cent water. This water with its dissolved salts and colloids fills the minute cell spaces and bathes all the tissues. It is the menstruum in which occur the chemical changes of the bodily processes. In its readily visible forms it is seen as blood and lymph. For normal functioning of the body the volume and composition of these circulating fluids must be kept fairly constant. This constancy is assured by the action of the kidneys in the production of urine. Blood, lymph, and urine can be profitably considered together. (Cf. Starling, *The Fluids of the Body*, 1909.)

I. BLOOD AND ITS PROPERTIES

The blood, pumped through the vessels by the heart, is the vehicle for carrying nutriment and oxygen to the countless myriads of cells situated remote from the sources of supply, and the vehicle also for carrying the waste from these cells to the organs by which the waste is discharged. The blood serves to protect the body against invasion by micro-organisms and against their toxic products. The blood is an important agency in maintaining chemical neutrality in the body. The blood is the means of coördinating such functions as are related by chemical agencies. The blood equalizes the distribution of heat, taking it from warmer regions and giving it to cooler regions, while rapidly passing through the vessels. The blood is clearly a very precious fluid, and knowledge of its properties is important.

The Blood as a Carrier of Gases

The body is very closely dependent on a continuous supply of oxygen, and suffers if there is an accumulation of carbon dioxide. Coöperating with the respiratory function of the lungs is the respiratory function of the blood. Through this coöperation the dangers of inadequate oxygen supply and inadequate carbon-dioxide removal are normally avoided. In addition to oxygen and carbon dioxide the blood carries a small amount of nitrogen — *i. e.*, the gases in the blood are the same as those in the air. These gases are surrendered by the blood when it is exposed to a vacuum in a blood-gas pump.

A blood-gas pump has as an essential feature a reservoir which can be filled with mercury, and after its connections have been tightly closed can have a vacuum developed within it by the withdrawal of the mercury. A simple apparatus of this character is that devised by Van Slyke (*Van Slyke, J. Biol. Chem.*, 1917, xxx, 347). It consists essentially of a 50 c.c. pipette with three-way cocks at top and bottom, and a 1 c.c. scale on the upper stem of the pipette divided into 0.02 c.c. divisions. Below the lower three-way cock is a tube and a chamber; the latter serves for drawing off fluids after the gases have been extracted from them. The tube and the chamber join below in a common tube to which heavy-walled rubber tubing is attached. This leads to a leveling bulb filled with mercury.

Determination of the CO₂ Dissociation Curve. — The CO₂ content of blood is to be determined when it is in equilibrium with gas mixtures (air) containing CO₂ at several different pressures. The per cent of CO₂ in the stock mixtures will be given by the instructor, or may be determined by analysis with the gas analyzer (see p. 132). Mixtures containing approximately 2.5 %, 6 % and 9 % CO₂ are recommended. The partial pressure of CO₂ may then be calculated from the formula,

$$\text{Partial pressure} = \frac{(\text{Barometric pressure} - \text{aqueous tension}) \times \% \text{CO}_2}{100}$$

Barometric pressure will be given out each day. Aqueous tension depends upon the temperature of the room, and may be obtained from the table on page 139.

Rinse the Van Slyke apparatus with distilled water to make sure that it is clean. See that the stopcocks are well greased and tight. Fill the burette with mercury; place about 1.5 c.c. of blood in the cup and draw the blood into the burette. Connect the outflow tube with the gas reservoir; draw in gas by lowering the mercury bottle until the mercury column has fallen to the bottom of the bulb; turn the upper cock and drive the gas out through cup, taking care, however, not to drive any blood into the cup. Refill with the gas mixture, *bringing the mercury column below the bottom of the bulb*; close the upper cock, bring the mercury in the bottle level with mercury in the analyzer, and close the lower cock. Shake the analyzer for 3 minutes. (If the mercury column is not set below the bottom of the bulb the shaking will produce a troublesome emulsion of mercury and blood. The purpose of leveling the mercury columns inside and outside the analyzer is to make sure that the gas mixture is at atmospheric pressure.) Drive the gas mixture out through the cup and refill as before. (Why change the gas?) Shake the analyzer again for 3 minutes. With gas mixtures containing less than 4 % CO₂ it is desirable to refill and shake a third time.

Place a drop of mercury in the cup and drive the gas in the bulb out through this trap by slowly raising the mercury bottle. Take care that the pressure inside never exceeds that outside by more than one centimeter. When most of the gas is expelled, very carefully decrease the pressure enough to draw the mercury drop into the bore of the upper cock. Then turn the cock and expel through the outlet tube the remainder of the gas and *all but exactly 1 c.c. of blood*. Wash out the cup with distilled water.

Now proceed as follows:

1. Place 1 c.c. of water in the cup. Under it place 2 drops of caprylic alcohol to prevent foaming.
2. Admit the contents of the cup into the burette, but do not allow air to enter the capillary at the bottom.
3. Run into the burette 0.5 c.c. of 10 per cent lactic acid and close the cock.
4. Evacuate the bulb by lowering the mercury nearly to the lower cock. Turn the cock. Shake the remnant of mercury with the blood for 3 minutes. Immediately read the volume of the gas in the manner demonstrated. (The lower cock is opened, the mercury in the leveling bottle is raised until it is above the level of the mercury in the burette by 1/13 of the column of laked blood.)
5. With a negative pressure of several centimetres allow 0.5 c.c. of 0.5 N of NaOH to run in and absorb the CO₂ that has been liberated.
6. Wait 2 minutes for drainage and read again the gas volume.
7. Wash the burette with distilled water.

The difference between the first and second readings is the volume of CO_2 in 1 c.c. of blood at the room temperature and the existing barometric pressure. To correct to standard conditions of 0° and 760 mm. barometric pressure, multiply by a factor obtained from the following table, which takes account not only of the effect of temperature and barometric pressure upon the gas volume but also corrects for the aqueous vapor present and for a small amount of CO_2 which remains unextracted or which is reabsorbed by the solutions during the analysis (Van Slyke and Stadie, J. Biol. Chem., 1921, xlix, 1).

Temperature ($^\circ\text{C}.$)	Factor	Temperature ($^\circ\text{C}.$)	Factor
15	$1.002 \times \frac{\text{Bar.}}{760}$	23	$0.954 \times \frac{\text{Bar.}}{760}$
16	0.995 "	24	0.948 "
17	0.989 "	25	0.942 "
18	0.983 "	26	0.936 "
19	0.978 "	27	0.931 "
20	0.972 "	28	0.924 "
21	0.966 "	29	0.918 "
22	0.960 "	30	0.912 "

The determination should be repeated several times with the same gas mixture until reasonable checks are obtained. Then repeat, using two other pressures of CO_2 . Plot the results with volumes per cent of CO_2 as ordinates and CO_2 pressures as abscissae. Draw a smooth curve from the origin and through the points as determined.

What would be the CO_2 content of the arterial blood if the partial pressure of CO_2 in the alveolar air is 40 mm. Mark this point "A" on your curve.

Calculation of the CO_2 in Solution and in Chemical Combination.—The volume of CO_2 dissolved by 100 c.c. of blood at a temperature of $38^\circ\text{C}.$ and at any partial pressure of CO_2 (p.p. CO_2) is given by the calculation; $100 \times 0.511 \times \frac{\text{p.p. CO}_2}{760}$ or $0.0672 \times \text{p.p. CO}_2$ (0.511 being the coefficient of solubility of CO_2 in blood).

This represents CO_2 as a dissolved gas and as carbonic acid. The remainder is present combined with base. Calculate the proportion of CO_2 in solution and in chemical combination with base at each of the pressures determined above. Draw a line on your graph indicating the volume per cent of CO_2 in solution at various pressures.

Calculation of the pH of the Blood.—The student is referred to textbooks of biological chemistry for a definition of the pH of the blood. It may be calculated as follows:

$$\text{pH} = 6.1 + \log \frac{(\text{Vol. \%CO}_2 \text{ combined with base})}{(\text{Vol. \%CO}_2 \text{ in solution})}.$$

Estimate the pH of the blood at each of the pressures measured above. Plot a curve relating the pH and the CO_2 pressure. What is the pH at the pressure of CO_2 in arterial blood (40 mm.)? in venous blood (about 50 mm.)?

Note.—The constants in the formulae are based on blood at $38^\circ\text{C}.$ Your results are consequently somewhat incorrect. The reduced O_2 content of venous blood also influences its pH, tending to minimize the difference between arterial and venous blood.

The Distribution of CO_2 between Corpuscles and Plasma.—Determine the CO_2 content of blood serum or plasma in equilibrium with about 40 mm. of CO_2 . For this purpose alveolar air may be used. Introduce about 4 c.c. of the serum or plasma into a dry separa-

tory funnel having a capacity of about 250 c.c. Breathe the last portion of a normal respiration into the funnel through a wash bottle or large test tube containing glass beads. The funnel is now filled with alveolar air (about 40 mm. of CO_2). Thereupon the funnel is turned in such a way as to spread the plasma in a thin film over the inner surface. After turning it thus for about a minute, another portion of alveolar air is introduced into the funnel and the turning is repeated. Thus the plasma is brought into equilibrium with the CO_2 tension of alveolar air. If care is taken not to warm the chamber through manipulation, the process may be regarded as occurring at room temperature.

One c.c. of the plasma is now introduced with a graduated pipette into the cup of the analyzer under 1 c.c. of water and 2 drops of caprylic alcohol and the analysis continued from step 2 as directed in the preceding exercise. The relative volume of corpuscles and plasma can be determined with the haematocrit. Estimate the relative quantities of CO_2 in the corpuscles and plasma as follows:

Vol. of CO_2 in 1 c.c. of whole blood — (fraction of plasma in whole blood \times vol. of CO_2 in 1 c.c. plasma) = vol. of CO_2 in the corpuscles of 1 c.c. of whole blood.

Vol. of CO_2 in 1 c.c. of whole blood — vol. of CO_2 in the corpuscles of 1 c.c. of whole blood = vol. of CO_2 in the plasma of 1 c.c. of whole blood.

The Influence of Acid and Alkali on the CO_2 Dissociation Curve. — Make the following mixtures:

5 c.c. of oxalated blood + 1 c.c. 0.8% NaCl
 5 c.c. " " " + 1 c.c. 0.1N HCl
 5 c.c. " " " + 1 c.c. 0.1N NaOH

The acid and alkali should be added slowly and with constant stirring.

Bring each mixture to equilibrium with CO_2 at approximately 40 mm. partial pressure and determine the CO_2 content. Alveolar air may be used as in the previous exercise.

Estimate the pH of the three solutions. The result illustrates the condition of the blood in uncompensated acidosis and alkalosis.

How may the three solutions be brought to the same pH? The answer may be worked out in principle by drawing dissociation curves through the three points and introducing a line representing the $\frac{\text{Combined } \text{CO}_2}{\text{Free } \text{CO}_2}$ ratio of the first sample. The points where this line cuts the other curves will give the conditions required. The condition of the blood in compensated acidosis and alkalosis are thus defined. How would the compensation be brought about in the organism?

The pH of Bicarbonate- CO_2 Solutions. — Bicarbonate solutions may be used to illustrate the general principle of buffering exhibited by a number of substances in the blood. The hydrogen ion concentration of a solution of a weak acid in the presence of its salt is expressed by the equation

$$C_H = K \frac{C_{\text{acid}}}{C_{\text{salt}}}$$

$$\text{Since by definition } \text{pH} = \log \frac{1}{C_H}$$

$$\text{pH} = K_1 + \log \frac{C_{\text{salt}}}{C_{\text{acid}}}$$

In the case of carbonic acid these equations become

$$C_H = K \frac{C_{H_2CO_3}}{C_{NaHCO_3}}$$

$$pH = K_1 + \log \frac{C_{NaHCO_3}}{C_{H_2CO_3}} .$$

From these equations it follows that doubling $BHCO_3$ should halve the C_H or increase the pH by 0.3.

Prepare solutions of the following in 5 c.c. amounts:

.01, .02, .04 M $NaHCO_3$.

Equilibrate the solutions with alveolar air.

Transfer the solutions to test tubes, add to each two drops of phenol red and compare the color with a standard series of buffers of known pH.

What effect will a proportionate change in CO_2 pressure and $BHCO_3$ have on the pH?

Prepare a series of dilutions of the .04 M $NaHCO_3$ to correspond with the gas mixtures provided — thus

.04 M $NaHCO_3$ — 12 % CO_2

.02 M $NaHCO_3$ — 6 % CO_2

.01 M $NaHCO_3$ — 3 % CO_2

Equilibrate and determine the pH colorimetrically as before. Do the results sustain the theory?

Boil distilled water to drive off the CO_2 and add phenol red. Now blow expired air through it, or equilibrate it with alveolar air. What is the effect on the pH?

The O_2 Capacity of Blood. — This may be defined as the volume of oxygen which may be extracted from 100 c.c. of blood when it is in equilibrium with atmospheric air. Place 2.5 c.c. of blood in the Van Slyke apparatus, draw in atmospheric air and shake the apparatus for 3 minutes. It is not necessary to change the air. Drive out the air and fill the outflow tube with mercury from the small mercury bottle. Turn the upper stopcock and run into cup all the blood in excess of *exactly* 2 c.c. Close the stopcock and rinse out the cup.

Proceed with the determination as follows:

1. Place in the cup 6 drops of 1 % saponin
2 drops of caprylic alcohol
6 c.c. of water.
2. Run into burette *all but* 1 c.c. Wait one minute for haemolysis to occur.
3. Add 3 drops of 20 % potassium ferricyanide to the contents of the cup and run it in.
4. Seal with a drop of mercury.
5. Evacuate the bulb and shake the analyzer for 3 minutes.
6. Absorb the CO_2 with 0.5 c.c. of C.5 N sodium hydrate.

7. Wait 2 minutes for drainage. Read.
8. Wash the apparatus with distilled water.
9. Subtract 0.115 from the reading for air dissolved in the reagent. Correct the remaining figure for nitrogen dissolved in blood, and for aqueous vapor, barometric pressure and temperature. (See Table 1.)

Calculate the per cent of haemoglobin.

Blood is said to contain 100 per cent haemoglobin when its O₂ capacity is 18.5 volumes per cent. The per cent of haemoglobin in the sample is consequently given by

$$\frac{\text{O}_2 \text{ capacity}}{18.5} \times 100.$$

TABLE 1
FACTORS FOR CALCULATING RESULTS FROM ANALYSIS OF 2 C.C. OF
BLOOD SATURATED WITH AIR¹

Temperature	Air physically dissolved by 2 c.c. of blood. Subtract from gas volume read in order to obtain corrected gas volume, representing O ₂ set free from haemoglobin	Factor by which corrected gas volume is multiplied in order to give oxygen chemically bound by 100 c.c. of blood
C.°	c.c.	c.c.
15	0.037	$46.5 \times \frac{B}{760}$
16	0.036	46.3 "
17	0.036	46.0 "
18	0.035	45.8 "
19	0.035	45.6 "
20	0.034	45.4 "
21	0.033	45.1 "
22	0.033	44.9 "
23	0.032	44.7 "
24	0.032	44.4 "
25	0.031	44.2 "
26	0.030	44.0 "
27	0.030	43.7 "
28	0.029	43.5 "
29	0.029	43.3 "
30	0.028	43.1 "

¹ A more comprehensive table is given by Van Slyke and Stadie (J. Biol. Chem., 1921, xlix, 1.)

The O₂ Dissociation Curve.—Gas mixtures will be provided containing a constant percentage of CO₂ and varying percentages of O₂. Mixtures of 6% CO₂ and 4.5% O₂ and 2.5% O₂ are recommended.

Calculate the partial pressure of each gas present in each mixture used.

In the Van Slyke apparatus equilibrate blood with these mixtures, taking care to change the gas mixture at least once as in determining the CO₂ dissociation curve. Seal the outflow

tube with mercury after admitting the final portion of the gas. Determine the O_2 content. Determine the oxygen capacity of the blood used, and estimate the percentage saturation of each sample, as follows:

$$\text{The percentage saturation} = \frac{\text{Content}}{\text{Capacity}} \times 100.$$

Plot the percentage saturation as ordinates against the O_2 pressure and draw a curve from the origin through the points.

What saturation corresponds to the O_2 pressure of alveolar air (100 mm.)? of mixed venous blood (35 mm.)?

The Effect of CO_2 on the O_2 Dissociation Curve.—A gas mixture will be provided containing a limited pressure of O_2 (about 2.5%) and no CO_2 . Determine the O_2 content of blood in equilibrium with it. Mark the corresponding point on the graph of the preceding exercise. What effect has CO_2 had on the affinity of blood for O_2 ?

The Coagulation of the Blood

One of the most important properties of the blood is its power of changing from a fluid to a jelly on escaping from the blood vessels. It is this property which closes a break in the vascular system and prevents such loss of the blood as might be dangerous to the life of the organism.

The Physical Changes in Coagulation of the Blood.—Etherize a cat which has been weighed, and introduce a vaselined cannula into the carotid artery. Continue the etherization during the period required for the following experiments. Release the clip from the vessel for a moment and fill with blood one third of a small test-tube containing absorbent cotton, and one-third of another small test-tube coated on the inside with vaseline. Note the changes that occur within ten minutes. In which tube does coagulation first occur? What further changes have occurred at the end of 24 hours? Note at this time the contracted clot and the expressed fluid—the serum.

Place a drop of fresh blood on a microscope slide and cover it with a cover-slip. Set over the slide a beaker lined with wet filter paper to prevent drying. From time to time examine the blood under the microscope for strands of fibrin. They should be clearly seen in fifteen or twenty minutes.

The Effect of Certain Neutral Salts.—In the observations on arterial pressure sodium citrate was used to connect the artery with the manometer. The reason for using this solution was that it checks coagulation of the blood. Other salts, such as sodium carbonate and magnesium sulphate, have the same effect.

Fill one fourth of a small test-tube with a saturated solution of $MgSO_4$; and add blood from the artery until the tube is three-fourths full. Wait 10 minutes, and note that the blood does not clot.

The Importance of the Intact Intima.—Lay bare 5 cm. of the external jugular vein of the animal from which the blood for the above experiment was obtained, and tie a ligature about the vein as near to the heart as possible. When the bared vein has filled with

blood, tie another ligature about it as far from the heart as possible. Note the time when this second ligature is tied.

Crush the vein between the second ligature and the head by strong compression with the artery forceps. After five minutes remove the forceps.

When the animal is dead examine the vessel in the region where the forceps were applied for an intravascular clot.

Open the isolated portion of the vein, and note again the time. Has the blood clotted in the vein?

How does the time of clotting outside the body compare with the time of clotting in a vessel with intact intima?

The blood supplied for the following experiments has been prevented from coagulating by the addition of sodium citrate, to make a 0.28 per cent solution with the blood. While experiment 1 is in progress, the separation of plasma and cells by centrifugalization will be demonstrated and material collected for experiments 2, 3, 4, and 5. The washed red cells used in experiment 3 have been obtained by centrifugalization, removal of plasma, substitution by salt solution, and repetition of the process. The serum of experiment 4 is pipetted from centrifugalized defibrinated blood.

The Importance of Calcium Salts. — 1. Place 0.5 c.c. of citrated blood in each of three test tubes. Add to one 3, to another 5, and to the last 7 drops of 1 per cent CaCl_2 noting the time when the mixture is made. Examine the tubes carefully at 30-second intervals by inclining them gently. How soon is coagulation evident?

How soon may the tubes be inverted without dislodging the clots?

Why does clotting occur?

2. Repeat experiment 1 using citrated plasma instead of whole blood. (Arthus and Pagès, *Arch. de Physiol.*, 1890, xxii, 739.)

Thromboplastin or Thrombokinase. — 3. Repeat experiment 1 using washed red corpuscles instead of whole blood.

Are the corpuscles an important feature in coagulation?

To 0.5 c.c. of washed corpuscles add 7 c.c. of distilled water. Shake well. What changes in appearance and transparency take place? Explain.

Add 5 drops of this *laked* blood to 0.5 c.c. of citrated plasma. Does clotting occur?

If it does not occur in 15 minutes, add the number of drops of 1 per cent CaCl_2 which you have found to give the quickest coagulation and record the time when clotting is complete. What has been the action of the laked blood? What factor of coagulation is contained in the corpuscles?

Thrombin. — 4. Add 0.5 c.c. of serum to 0.5 c.c. of citrated plasma. Does clotting occur?

What factors of coagulation does the serum contain?

Repeat the experiment with serum heated to 60° C.

Antithrombin. — 5. By heating citrated plasma to 60°C., its fibrinogen and prothrombin are removed. Antithrombin is left. Demonstrate the presence of antithrombin by the follow-

ing procedure. As shown in experiment 3, coagulation will result if 5 drops of laked blood and then 5 drops of 1 per cent CaCl_2 are added to 0.5 c.c. of citrated plasma. Add also a few drops of the heated citrated plasma and note whether a clot forms. (See Howell, *The Harvey Lectures*, New York, 1916-17, p. 272.)

***The Prevention of Coagulation by Intravascular Injection.**—Inject into a vein of a dog or cat a solution of albumoses (0.5 gm. albumose per kilo body weight). In a few minutes fill one third of a small test-tube with blood from the animal. Is there any change in the coagulation time?

***The Counting of the Corpuscles**

“Blood-counting” is of value in giving an idea of the amazing number of corpuscles normally present in the blood, and also in showing the method of estimating the number of corpuscles per unit volume in abnormal states. The most satisfactory apparatus for enumerating the corpuscles is the Thoma-Zeiss haemocytometer, which consists essentially of a counting chamber of known volume, and pipettes for accurate dilution of the blood. There are two pipettes; one with a larger capillary and a smaller bulb than the other. This tube is arranged for a dilution of 0.5 or 1 to 10, and is used in the preparation for counting white corpuscles. The tube with the smaller capillary is arranged for a dilution of 0.5 or 1 to 100, and is used for red corpuscles. Two counting slides are provided; thus one person may be counting the white corpuscles while another is counting the red. Before starting the enumeration be sure that the counting slides and the pipettes are clean and dry. The small glass bead in the bulb should not stick to the walls. (See Malassez, *Arch. de Physiol.*, 1874, vi, 32.)

Drawing the Blood.—Secure from the ear the blood for counting. The ear is less sensitive and under the same conditions gives more blood than the finger. With absorbent cotton moistened in distilled water wash the lobe of the ear gently. Dry the surface with another piece of cotton. Hold the lobe of the ear between the thumb and finger, and with the other hand quickly thrust a three-sided needle about a quarter of an inch into the edge of the lobe. At once withdraw the needle, but in doing so give it a half turn. Wipe away the first drop (it coagulates faster than the later blood), and prepare to take the second drop as it oozes from the puncture. Never squeeze the blood out, as it is then not normal. The ear may, however, be gently manipulated 2 or 3 cm. above the puncture. Do not attempt to draw the blood into the pipette until a large drop has formed.

Counting the Red Corpuscles.—The blood is drawn into the pipette by suction. Hold the lobe of the ear gently between the thumb and finger. Steady the tip of the “red counter” against the thumb, which is placed behind the ear, while the pipette is held horizontally near the bulbar end in the other hand. Cautiously insert the point into the drop of blood, but do not bring it in contact with the skin. By very careful suction fill the capillary to the line marked 0.5. If this point is passed and there is enough blood, fill to the next division. The filling must be accurate. If the tube is not filled accurately and completely to one line or another, blow the blood out, clean the pipette according to the directions at the end of this exercise, and try again to secure an accurate quantity of blood in the capillary. With absorbent cotton wipe the end of the pipette free from blood, but in this

process do not withdraw any blood from the tube. As soon as possible (why?), dip the end in a beaker of Hayem's diluting solution.¹ Suck up a continuous stream of this solution until the point 101 is reached (why 101 instead of 100?). As the blood enters the bulb, roll the pipette between the thumb and finger.

Now hold the pipette horizontally, close the lower opening with the finger, and mix the solution and blood in the bulb by gently shaking it to and fro for several minutes. The bulb now contains 0.5 or more of blood in 100 parts of the mixture.

Blow out the contents of the capillary (why?), and reject the first few drops of the mixture. Place a small drop on the counting slide, just enough to fill the space between the marked surface and the cover-glass without spilling into the moat. If it spills, clean the disc and use a second, smaller drop. Adjust the cover-glass by placing one edge in contact with the slide and letting the other edge down carefully with a needle. Now set the counting chamber on the microscope stage, and after waiting a few minutes for the cells to settle, begin the counting. In doing this use a Leitz objective 5 or 6, or a Zeiss D. Stronger objectives cannot be employed with the thick cover-glasses of the instrument. On the central part of the disc are ruled two sets of 21 parallel lines at right angles to each other and exactly $1/20$ mm. apart. Thus a square millimetre is divided into 400 small squares. An extra line ruled through every fifth row forms a double row of rectangles around 16 of the small squares. Find a block of 16 small squares. Begin counting in the upper left-hand small square in the block; count all the corpuscles in the square and those on the upper and left-hand border lines. Next count in the same manner the corpuscles in the space below. Continue thus until the four squares in the row are counted. Count likewise the squares in the next row, and so on until all 16 are counted. As each square is finished, set down the number of corpuscles found within it. For accuracy not less than 1200 corpuscles should be registered. To avoid repetitions go over the larger squares in the same orderly manner in which the small squares are studied.

The depth of the counting chamber is $1/10$ mm.; the area of each small square is $1/400$ sq. mm.; the volume of fluid above each square is therefore $1/4000$ cu. mm. One cubic millimetre would contain 4000 times the average number of corpuscles found on a single square. If the dilution of the blood was 100, this number must be multiplied by 100; if 200, by 200. Calculate from the number counted the average number of corpuscles per square, and estimate the number per cubic millimetre of blood.

Repeat the count with a second drop; if the two countings do not closely agree, try a third.

Counting the White Cells.—The same method is employed in counting leucocytes as in counting red corpuscles, except that the pipette giving the lower dilution is selected, and 0.5 per cent glacial acetic acid, which dissolves the red corpuscles, is used as a diluting fluid. The large bore of the capillary in the pipette used in the leucocyte count requires more blood than is needed for estimating the red corpuscles; attempt to draw the blood into the tube, therefore, only to the line 5. This large bore also permits the fluids to run in or out easily; for that reason take care to hold the tube horizontal. Follow the directions given for the counting of the red corpuscles, in mixing the blood and the diluent, placing the mixture on the counting disc, and setting the cover-glass.

¹ NaCl 2, Na₂SO₄ 10, HgCl₂ 1, H₂O 400. Another serviceable fluid is Kemp's:—1 per cent NaCl 150, 40 per cent formaldehyde 10.

In counting, a low-power objective may be used, and the white cells in a whole square millimetre seen at once. Count the number in the entire ruled field, and calculate the number per cubic millimetre. As there is a greater liability of error in enumerating the white cells than the red corpuscles, the procedure may well be repeated.

Cleaning the Instruments.—Always leave the instruments scrupulously clean. When the pipettes are ready to be put away blow out their contents. Draw into them hydrogen peroxide which oxidizes any blood that may be adherent to the walls, follow the hydrogen peroxide with distilled water, and dry the pipette with alcohol and finally with ether. Do not begin the cleaning with alcohol and ether; they coagulate the albumin of the blood. Draw the alcohol from the filled pipette into the rubber tube, remove the tube, blow out the alcohol, replace the tube, and draw air through the pipette. Then do likewise with the ether. Why must these drying fluids not be blown out of the pipette?

Follow the same order in cleaning the cover-glass as in cleaning the pipettes.

The disc on the counting slide is fastened by a cement soluble in alcohol and ether. Do not therefore use alcohol or ether in cleaning the disc. Wash the disc with distilled water, and dry with a soft cloth or absorbent cotton. Do not wipe vigorously. If necessary soap and water may be used, but the glass must afterwards be thoroughly rinsed.

The Estimation of Haemoglobin

The capacity of the blood for oxygen is chiefly dependent on the content of haemoglobin. Haemoglobin is bound in the red corpuscles; in order to estimate it a standard amount of blood must be taken and the haemoglobin caused to leave the corpuscles and become dissolved. This process is known as "laking" the blood (see p. 110).

The Colorimetric Estimation of Haemoglobin.—Laked blood is colored by the haemoglobin which is in solution. Haldane has shown that there is an exact relation between the color produced by a standard volume of blood and its capacity to carry oxygen. It is only necessary to have a standard color of laked blood corresponding to a certain oxygen capacity, and the oxygen capacity of any sample of blood can be tested by matching the colors. On these facts is based the use of the Gowers-Haldane haemoglobinometer (Haldane, *J. Physiol.*, 1901, xxvi, 497). This instrument provides a quick, convenient method for determining the oxygen capacity of blood, in detecting such conditions as anaemia, and in following the dilution of the blood as the result of experimental procedures. As oxyhaemoglobin is unstable, the haemoglobin is combined with carbon monoxide, and a fairly permanent standard solution thus secured.

The standard tube is supposed to contain 1 per cent of ox blood in a solution saturated with coal gas (the coal gas contains CO). The oxygen capacity of the blood used for the standard is 18.5 volumes per cent, which corresponds to about 13.8 per cent haemoglobin in the total volume of blood. If the standard amount of human blood saturated with CO and diluted with water to the mark 100 in the graduated tube corresponds to the normal tint, it is normal and has therefore an oxygen capacity of 18.5 volumes per cent. The haemoglobin is then said to be present to the amount of 100 per cent. If the tint matches the standard at less than the dilution to 100, the percentage ratio of the tested blood to the normal can be directly read.

As the standard solution is difficult to obtain, and is not invariably stable, it is simplest to prepare a standard by diluting blood of known capacity to 1 per cent with distilled water — 1 c.c. in a 100 c.c. flask — and estimating a factor by which the readings must be multiplied in order to correct for the actual O₂ capacity of the solution employed. The factor is given by

$$\frac{\text{O}_2 \text{ capacity}}{18.5}.$$

Determine the O₂ capacity of a given blood, make a 1 per cent solution, saturate it with illuminating gas, fill the ungraduated tube in the Gowers-Haldane outfit and cork it tightly. Calculate the factor and keep the figure for future use.

Fill the graduated tube with distilled water to the mark 20. Use the methods for securing blood described in the directions for enumeration of blood corpuscles, and take the same precautions to have all instruments clean. Draw into the pipette exactly 20 cu. mm. of blood. Wipe off with absorbent cotton any blood that may be adherent to the point or sides of the pipette. Insert the pipette into the graduated tube almost to the water and gently blow out the blood drop by drop. Immediately shake the tube to mix the water and blood. Since blood still remains on the inner surface of the pipette, fill the pipette with distilled water and blow this also into the graduated tube. Repeat the procedure three or four times.

Connect a small glass tube with a gas-tap, turn on the gas, and push this tube into the graduated tube nearly to the level of the water. As soon as the gas has displaced the air, withdraw the gas-tube, quickly close the graduated tube with the finger, and turn off the gas. Invert the graduated tube about a dozen times until the haemoglobin is thoroughly saturated with CO. The solution becomes pink from CO-haemoglobin. Avoid any loss of the colored fluid as the finger is removed.

Add distilled water drop by drop to the solution until the tint appears equal to the standard. After a half minute for the liquids to mix read the percentage. In comparing the tints look through the solutions towards the sky, and, in order to avoid error, frequently transpose the tubes during the observations. After the tints appear equal add more distilled water drop by drop until the tints appear just unequal. Again read the percentage. The mean of the two readings is taken as correct. The average haemoglobin percentage in women is 11 per cent and in children is 13 per cent below that of adult men.

Repeat the observation with the instrument several times. The results with the same blood should agree within 1 per cent of the mean.

Clean the pipette according to the directions given at the end of the exercise on blood counting.

***Haemolysis**

The important rôle which the blood plays in protecting the body against organic invaders and their toxic products has already been noted. A power of disintegrating bacteria can be developed in the blood of an animal by injecting cultures of bacteria; an antitoxin can be produced by injecting a toxin. This most interesting and important protective reaction of the organism can be illustrated by a repetition of some of Bordet's experiments on the effects of injecting an alien blood. The serum of some animals will cause laking of the red corpuscles of other animals; such is the relation between the blood serum of the cat and the red corpuscles of the rabbit. Guinea pig serum, however, will not normally

disintegrate, or "haemolyse," rabbit corpuscles. If, however, defibrinated rabbit blood has been injected two or three times into the guinea pig which yields the serum, the serum will then actively haemolyse the rabbit corpuscles. The haemolysis, whether natural or artificially developed, is preceded by a clumping of the corpuscles, known as "agglutination." (Bordet, *Ann. de l'Inst. Pasteur*, 1898, xii, 692.)

Preparation for Bordet's Observations. — Two weeks before the observation on haemolysis is to be made, a guinea pig is injected subcutaneously with 3 or 4 c.c. of defibrinated rabbit blood which has been obtained painlessly and with aseptic precautions. One guinea pig will serve for 12 students. The injection should be repeated twice at intervals of 3 days.

On the second day before the observation the guinea pigs are etherized and bled into a conical vessel. Into another conical vessel one normal guinea pig is similarly bled for each group of 24 students. Into a third vessel a cat is bled. The clots are permitted to contract in a cold place for 36 hours and press out the serum.

On the day before the observation a rabbit is bled and the blood defibrinated. This blood is centrifugalized and the clear serum saved. The corpuscles are then washed three times by centrifugalizing them in mammalian Ringer's solution. After this process is ended a mixture of 5 parts corpuscles and 95 parts Ringer's solution is made and set in the cold until the next day.

On the day of the observation the serum from the blood of the injected guinea pigs (the so-called "immune serum"), the normal guinea pig serum, and the normal cat serum are poured off. More serum can be secured in each instance by centrifugalizing the clot. Half of the immune serum is heated at 55° C. for 30 minutes; and half of the cat serum is heated to 60° for 15 minutes. Each group of 24 students is now supplied with 7 small bottles containing immune guinea pig serum, immune guinea pig serum heated to 55° C. for 30 minutes, normal guinea pig serum, rabbit serum, washed rabbit corpuscles, normal cat serum, and cat serum heated to 60° for 15 minutes. Each bottle must have its own capillary pipette. Each pair of students receives a small block holding 7 test-tubes about 2 cm. high made of glass tubing with an inside diameter of 5 mm.

By use of a microscope and the test-tubes make the following observations:

Agglutination. — Mix equal parts of normal guinea pig serum and washed rabbit corpuscles in the first test-tube, and let the mixture stand.

Place a small drop of each of these fluids on a microscope slide; drop on them a cover-glass, and note the clumping of the corpuscles in the region where the two drops mingle.

This clumping causes the corpuscles in serum or Ringer's solution to sink much more rapidly than they would sink separately. Observe from time to time the first test-tube.

Agglutination and Haemolysis. — Mix in the second test-tube immune serum and washed rabbit corpuscles (1 part to 3). Note the opacity of the mixture. In a short time examine the test-tube again. How has the appearance changed?

What is the inference regarding the haemoglobin?

Look at two drops of these fluids run together under a cover-glass. Does agglutination still occur?

Look carefully at an individual red corpuscle. After a time it loses its color and becomes hardly visible.

The Effect of heating Immune Serum to 55° C. — Mix immune serum heated to 55° C. for a half hour, and washed corpuscles (1 part to 3). After the test-tube has stood for some time compare its appearance with the first and second tubes. Also make a comparison under the microscope. How has the heating changed the immune serum?

The Effect of adding Normal Serum to Immune Serum Heated to 55° C. — Mix in a fourth test-tube immune serum heated to 55° C. for a half hour, washed rabbit corpuscles, and normal rabbit or guinea pig serum. After giving time for the changes to occur, compare the contents of this tube with the contents of the first three tubes. If the heating to 55° C. for a half hour destroyed some factor in the production of haemolysis, it was evidently not the new factor which the rabbit blood developed in the guinea pig, but something existing normally in the guinea pig blood. The addition of the normal serum has "reactivated" the heated serum.

Natural Haemolysis. — Mix in the fifth test-tube washed rabbit corpuscles and cat serum. What is the effect?

Does cat serum cause agglutination of the rabbit corpuscles?

The Effect of heating Cat Serum to 60° C. for 15 Minutes. — With cat serum heated to 60° C. for 15 minutes repeat the foregoing experiment. How has the heating changed the action of the cat serum on the rabbit corpuscles?

Some of the effects in the above experiments may show best after the mixtures have stood several hours.

II. LYMPH AND LYMPH FLOW

The function of the circulatory system, as already noted, is to maintain a continuous slow movement of the blood through the capillaries. There the functions of the blood with reference to the tissues are performed. The blood in the capillaries, however, does not come in contact with the tissue cells. To conduct the interchange between the blood and the tissue cells a medium is required. This medium the lymph supplies, — bearing to the cells nutrition and oxygen, and bearing away from the cells the waste resulting from their activity. The lymph is formed by the passage of a portion of the fluid part of the blood and some of the leucocytes through the capillary walls into the perivascular spaces. Lymph, therefore, may be regarded as a modified plasma plus leucocytes. The degree of its variation from the blood plasma differs in different organs of the body.

Lymph Production

Normally the lymph flows away as rapidly as it is formed. As it flows it is gathered into larger and more definite lymph channels, and is finally restored to the blood again in veins near the heart. Thus a circulation of the lymph is maintained. If lymph is poured through the vessel walls more rapidly than it flows away, an accumulation results. This condition is seen in the swelling produced by a blow, in the production of a blister, and in any state of inflammation. As lymph production is best observed in these exaggerations of the normal process, they will be utilized in the study.

***The Extravasation of Lymph in Inflammation.** — The following phenomena, observed and carefully described by Cohnheim, are the typical changes in the lymph flow in a condition of inflammation (Cohnheim, *Virchow's Arch.*, 1867, xl, 1; 1869, xlv, 333).

Inflammation in the Mesentery. — Anaesthetize a frog with urethane, as directed on page 27. Make a lateral opening in the abdominal wall, draw out some of the intestine, and lay the mesentery carefully over the window on the mesentery board. Be careful to avoid stretching and drying of the tissues. The exposure and handling of the mesentery results in inflammation. With a microscope observe closely the changes that occur in the blood vessels.

First there is dilatation of the arteries, veins, and capillaries, and an increased rate of flow, especially in the arteries. The acceleration does not last; in a half hour or more, occasionally in less time, it gives way to retardation. As the stream becomes slower, individual corpuscles can be seen not only in the capillaries, but also in the veins, and even in the arteries during diastole. Many of the capillaries are now crowded with corpuscles.

Slowly and gradually a characteristic change occurs in the veins; the "plasmatic zone" becomes filled with many leucocytes. The leucocytes remain almost motionless, while the red corpuscles move on in a continuous stream. This separation of the white from the red corpuscles is not seen in other vessels than the veins.

Now by careful observation an interesting sight may be observed. Usually in a vein, but sometimes in a capillary, a pointed projection of the vessel wall can be seen. The projection grows to a colorless rounded lump, which becomes larger and thicker, and gradually withdraws itself from the wall until it is connected only by a long thin pedicle. Finally the pedicle is detached and a contractile corpuscle, with one or more nuclei, lies outside the vessel. Thus do the leucocytes pass from the vessels into the lymph spaces. The emigration may occur early or may be retarded; but after 6 or 8 hours every vein will be covered with several layers of leucocytes. From the capillaries not only white corpuscles but red corpuscles as well will pass out as the inflammation continues.

Along with the emigration of the leucocytes there is an extravasation of lymph into the meshes of the mesentery. The mesentery consequently swells. When the emigrated leucocytes, and the extravasated lymph and red corpuscles can no longer be accommodated in the tissues, they pour out on the surface of the mesentery. Usually the lymph coagulates on the surface, making a fibrinous layer enclosing numerous leucocytes and isolated red corpuscles.

These are important observations for the understanding of inflammation, and should be patiently followed.

Inflammation in the Frog's Tongue. — Accompany the observation on the frog's mesentery with an observation on the frog's tongue. Withdraw the tongue from the mouth of a curarized frog with cerebrum pithed, and place it over the window in a mesentery board. Paint a part of the smooth surface of the tongue with croton oil diluted (1:10) with olive oil. After a minute or two wipe off the oil. Study the stages of the process of inflammation under the microscope. As the tissues become reddened, to what is the redness due?

***The Extravasation of Lymph in Active Congestion.** — Cohnheim observed that if the blood vessels of warm-blooded animals are exposed to unduly high or low temperatures a series of changes occur which could be best explained as the result of internal changes in the vessel wall.

Select a rabbit with a white ear. Interrupt the blood flow in the ear by placing a rubber band around the base. Hold the tip of the ear in warm water until it has a temperature of 48°C. After exposure to this temperature for a few minutes free the ear from the rubber band.

When the time required for the changes has passed, compare the warmed ear with the normal. Is there swelling?

Look through the ears towards the light; if swelling is present, is it due to increased size of blood vessels?

Compare the temperature of the two ears.

Which is the redder? To what is the redness due?

***The Extravasation of Lymph in the Human Skin.**— Wash a small area on the forearm. When it is dried place a small drop of strong ammonia on the surface and hold the arms steady for about 5 minutes. A small blister will form under the ammonia. With what is the blister filled, blood or lymph?

The Absorption and Flow of Lymph

The lymph which passes through the capillary walls into the perivascular spaces may flow away in lymph channels and thus be restored to the blood, or it may, in certain conditions, be restored directly to the blood by passing back through the capillary walls. It is difficult in any special case to tell to what degree each of these processes is concerned, but the two possibilities should be kept in mind.

***The Passage of Fluids from the Lymph Spaces of the Frog.**— Already in observations on the action of curare and strychnine the taking up of solutions from the dorsal lymph sac into the general circulation of the frog has become a familiar fact. The rapidity of the process and the general diffusion of the solution throughout the body can be seen in the following experiment.

Pith the brain of a frog without loss of blood. Make a small incision in the skin of the leg, and by means of a fine pipette introduce a few drops of indigo-carmin. After 4 or 5 minutes, during which the observations suggested in the next experiment may be made, open the abdomen at one side of the middle line, in order to avoid the anterior abdominal vein, and expose the viscera. Report what is observed.

***Lymph Hearts in the Frog.**— While waiting for the completion of the foregoing experiment, examine carefully in strong light the dorsal surface on either side of the posterior end of the urostyle. The pulsation which is seen is due to lymph hearts, which pump the lymph into neighboring veins. What is the rate of the rhythm? Is it uniform? Does it bear any close relation to the heart rate?

After opening the abdomen, as directed in the foregoing experiment, dissect away the skin overlying the lymph hearts, with care not to cut too far laterally, thus avoiding injury to a cutaneous vein. Describe the form and size of the lymph hearts. Pith the spinal cord. What now is observed? What may be inferred?

***Relative Speed of Absorption of Subcutaneous and Intramuscular Injections.**— Let each group of students make observations on the heart rate, the respiration rate, the posture, and the activity of rabbits after the injection of morphine.

Weigh each rabbit. Inject 8 mgm. of morphine sulphate per kilo into each animal. Let half the animals receive the morphine subcutaneously, and let the other half receive it in the muscles of the back in the lumbar region. At 5-minute intervals for 40 minutes record the results. Compare the results of intramuscular and subcutaneous injection. (Meltzer and Auer, *J. Exp. Med.*, 1905, vii, p. 59.)

(*Demonstration. — Absorption from Liver Lymph Channels.* — The bile secreted by the liver is reabsorbed, if the pressure in the bile capillaries is increased. By ligature of the thoracic duct it has been proved that the bile reaches the blood by way of lymph vessels.

A white rabbit is etherized and a cannula placed in the common bile duct. With the cannula is connected a small vertical tube containing normal salt solution to the height of about 5 cm. The height at which the bile pressure is registered is carefully noted. The tube is now replaced with a burette containing a filtered solution of indigo-carmin in isotonic salt solution. The height of the fluid in the burette is only a few centimetres above that reached by the bile. The indigo-carmin is permitted at once to flow into the bile duct.

In 5 or 10 minutes examine the edges of the eyelids and the lips and note the blue color due to the absorption of the indigo-carmin and its distribution by the blood. Increase the etherization until death ensues. Open the abdominal cavity and examine the viscera for the coloring matter. Note how slight the pressure of the bile need be to lead to absorption from the bile capillaries — how readily therefore jaundice may be produced. (Heidenhain, *Studien d. physiol. Inst. z. Breslau*, 1868, p. 233.))

(*Demonstration. — The Flow of Lymph from the Thoracic Duct; the Action of a Crystalloid Lymphagogue; the Coagulation of Lymph.* — About three hours before the demonstration a hungry cat is fed a meal consisting of finely chopped fat meat. The cat may be permitted to drink some cream about an hour before the demonstration.

The animal is fully anaesthetized with ether. By a longitudinal incision the muscles from larynx to sternum are laid bare, and a tracheal cannula is inserted. The pectoral muscles are next severed along their anterior origins close to the sternum. The posterior half of the left sterno-thyroid muscle is now removed, and the large veins of the left side thus exposed. With a blunt dissector the internal and external jugular and the subclavian and innominate veins are separated in the region of their union. A moist ligature is tied about each jugular vein and about the subclavian. Two ligatures are tied about the innominate as deep in the chest as possible, and the vein severed between them. The innominate vein is now lifted by the anterior ligature, and a cannula tied into the vein some distance from the opening of the duct. The cannula is connected with a glass tube ending in a fine point beyond the animal's shoulder. The lymph drops on the end of a light lever, the other slightly heavier end of which bears a strip of white paper, visible to the entire class. From the lever the drop falls into a narrow beaker. Each drop is thus signalled by the rise and fall of the white strip. The students should determine the number of drops per minute.

Into the jugular or crural vein, which has previously been provided with a cannula, about 20 c.c. of 100 per cent solution of glucose is now slowly introduced. Record the number of drops per minute for the 10 minutes following the injection. What is the effect of the injection of the glucose?

After the lymph has stood for some minutes, pour off the uncoagulated portion and observe the clot in the bottom of the beaker.)

III. URINE

The function of the kidneys is to maintain the composition of the circulating fluids in a fairly constant state. A very slight excess of urea, or sodium chloride, or water, or acid, or alkali, or percentage of sugar in the blood will induce a compensatory excretion of these substances. On the other hand, a salt free diet results in practical disappearance of chlorides from the urine, though in the blood they remain abundantly present.

Maintenance of the Composition of Blood after the Ingestion of Water. — Three members of each group will act as subjects. Breakfast should be omitted. The subjects should note the time of emptying the bladder in the morning and collect hourly samples of urine thereafter. At 10 o'clock determine the haemoglobin in the blood. Each subject should then drink one of the following as rapidly as he can without discomfort.

1. 1000 c.c. of water
2. 1000 c.c. of water + 10 gr. NaCl
3. 100 c.c. of water + 10 gr. NaCl

During the next 2 hours and a half make urine collections and haemoglobin determinations every half hour, and after that every hour until the rate of secretion has fallen to that of the preliminary control period. Measure the volume of each collection and determine its chloride content, using the method outlined in biochemistry. (Standardized reagents will be supplied to each group from the stock room.) Express your results in terms of *c.c. of water per hour* and *grams of NaCl per hour* during each experimental period and plot the results of each experiment. Also plot on one chart the rate of water excretion in each subject; and on another chart the rate of chloride excretion.

How long is required before all the ingested water and salt is removed from the body?

Which solution causes the least departure from the control conditions of secretion?

Does the concentration of haemoglobin vary as the result of water or salt ingestion? How do you interpret this result?

What evidence does the experiment offer as to whether salt or water can be stored in the body as fat and sugar are?

Does water diuresis carry out from the organism salt which would not otherwise have been excreted?

Does salt diuresis cause an excess of water to be excreted?

Calculate the concentration of chlorides — as NaCl — in the urine secreted in each case. In which cases does the kidney do osmotic work in producing the urine?

Conditions Affecting Excretion of Urine. — The cat provided has been anæsthetized with urethane (2 gr. per k.) introduced into the stomach in aqueous solution. The four students who will perform the experiment will be designated as Operator, Assistant, Kymograph Manipulator, and Manager of Stimulation and Injection. Their duties are the following: —

The Operator shall see that the experiment is conducted *expeditiously* and in a systematic fashion in accordance with the directions, and shall keep in writing a thorough record of the entire experiment. He should perform or supervise the following operations; insertion of a tracheal

cannula, insertion of a cannula in the femoral vein for injection, insertion of a cannula in the right carotid artery for recording blood pressure, exposure and cutting of the left vagus nerve and arranging for peripheral stimulation (see experiment on Blood Pressure in the Cat); exposure of the urethra, insertion of a long slender glass cannula through an opening in it into the bladder and tying it in place. The Operator shall also open the body cavity and pass ligatures about the renal vessels, but he should not undertake this operation until the time indicated.

The Operator's Assistant shall see that the operator is provided with the following instruments: heavy scissors, heavy forceps, fine scissors and forceps, blunt dissector, tracheal cannula, two arterial cannulae, one urethral cannula, ligature thread, and absorbent cotton. During the experiment he records the flow of urine by pressing the key of the signal magnet every time a drop falls from the urethral cannula.

The Kymograph Manipulator is responsible for the operation of the kymograph and washout system. He prepares the mercury manometer to record the carotid pressure, sets below the writing point a time signal and a signal magnet operated by a key, and has ready a saturated solution of Na_2CO_3 .

The Manager of Stimulation and Injection provides himself with the following articles: — an induction coil and electrodes arranged to stimulate the vagus nerve, a burette, a syringe, adrenin (1:10,000), urea (1 per cent), and NaCl (0.9 per cent). He is responsible for carrying out the following procedures.

Do not attempt to repeat these observations, because the secretion will not continue indefinitely.

The Effect of Blood Pressure. — Determine the normal blood pressure and rate of urine flow. Inject 0.5 c.c. adrenin (1:10,000) into the femoral vein.

What is the effect on blood pressure? on diuresis?

How can these effects be correlated?

Stimulate the vagus nerve.

What is the effect on arterial blood pressure; on diuresis?

The Effect of the Chemical Composition of the Blood. — Inject into the femoral vein 15 c.c. of a 1 per cent solution of urea.

What is the effect on diuresis?

Can this effect be attributed to changes in blood pressure?

After the urine flow returns to normal, inject 50 c.c. of a warm isotonic salt solution *slowly* into the femoral vein.

What is the effect on diuresis?

Can this effect be attributed to changes in arterial blood pressure?

The Time Interval for Urine Excretion. — Introduce 5 c.c. of a saturated solution of indigo-carmin into the vein. Note the time which elapses until the blue color first appears in the urine.

Asphyxiation of the Kidney. — For 60 seconds close the tracheal cannula with the finger.

Note and explain the effect upon diuresis.

Can this be explained by the changes in arterial blood pressure?

The operator may now open the body cavity and place ligatures around the renal arteries and veins of both kidneys and around the aorta caudad to the renal arteries. Look for rhythmic movements of the ureters.

Close the aorta below the renal arteries.

State and explain the effect on diuresis.

Close the renal veins by pulling up on the ligatures about them. Do not check the renal circulation for more than a minute in this way.

State and explain the effect on diuresis.

Close the renal artery by pulling up on the ligatures for 3 minutes.

What is the effect upon diuresis? Explain.

Does the kidney recover from the asphyxiation?

(*Demonstration. — The Circulation in the Glomeruli of the Kidney.* — An 8 mm. hole is bored into a suitable frog board, and a snugly fitting glass tube, sealed at one end, is pushed through the hole so that the sealed end protrudes about 8 mm. above the surface of the board. A male frog is anaesthetized with urethane (0.03 c.c. of a 10 % solution per gm. of frog injected into a lymph sac). A longitudinal incision to admit the sealed end of the glass tube is made then through the skin and muscles of the back along the lateral side of the cephalic end of the os coccygis. This incision is at the level of the kidney. It should be made with care to prevent haemorrhage and injury to the underlying kidney and peritoneum. For convenience, the cephalic end of the ilium (lateral to the incision) may be cut through or removed.

A second longitudinal incision is made then through the anterior abdominal wall lateral to the anterior abdominal vein. By pushing the underlying viscera aside gently, the kidney is brought into view at the side of the vertebral column. Haemorrhage and injury to blood vessels should be avoided as far as possible.

Finally the frog is placed back down on the board, so that the sealed end of the glass tube presses up through the incision in the back against the dorsal surface of the kidney. The preparation is placed on a microscope stage, and the kidney is illuminated by a strong beam of transmitted light with the red rays filtered out. The position of the kidney may be adjusted properly by placing tension on the peritoneum adjacent to it. Under the low power of the microscope, the glomeruli may be seen as rosettes of capillaries lying in the lateral margin of the kidney between the branches of the renal portal vein. Note whether the glomeruli collectively and whether all the capillaries in a single glomerulus are continuously active. Filling the abdominal cavity with normal salt solution will improve the circulation through the glomerular loops. (Richards, Amer. J. Med. Sci., 1922, clxiii, p. 1.)

METABOLISM

As observed in experiments on the flow of lymph from the thoracic duct and on the absorption of fat, fat is carried from the intestine into the blood stream by way of the lacteals and the thoracic duct. Products of protein and carbohydrate digestion are taken up directly by the blood vessels in the villi, and pass by the portal system through the liver before reaching the general circulation. Fat and carbohydrate are ultimately oxidized, and their waste eliminated as carbon dioxide and water. Protein undergoes complex changes in the body, some of them synthetic in character, and its waste is eliminated as carbon dioxide, water and as the urinary compounds containing nitrogen.

Knowledge of the oxygen, carbon and energy exchanges of the organism is as important in many cases as knowledge of the nitrogen exchange revealed by urine analysis, and in clinical laboratories determination of the oxygen intake and the carbon output is becoming as much a matter of routine as the nitrogen determination. The respiratory exchange is thus used to interpret changes in body weight, to discover whether a decrease is due to the loss of fat, muscle, or carbohydrate, to learn whether the demand for nutriment as indicated by the materials oxidized in the organism is met by the supply, as well as for other important considerations. Furthermore, analysis of the respiratory gases yields information regarding the composition of alveolar air and the delicacy of its control, and regarding the CO_2 and O_2 content of the blood both venous and arterial. After the principles of the respiratory exchange of the organism have been learned by means of gas analysis, simpler methods of determining the metabolic rate in the body can be employed with clear understanding.

The Gas Analyzer.—The type of apparatus employed in the following experiments is shown in Fig. 10. (See Henderson, Y., *J. Biol. Chem.*, 1918, xxxiii, p. 337.) The reare four main parts. **A.** A carefully graduated and calibrated burette for measuring the volumes of the samples. **B.** A temperature-pressure control tube. **C.** An NaOH chamber for CO_2 absorption. **D.** A potassium pyrogallate chamber for O_2 absorption. The burette and the temperature-pressure tube are connected at their lower ends by a Y-tube to a leveling bottle which contains acidulated alcohol (1 per cent H_2SO_4 in 40 per cent alcohol). By changing the height of the leveling bottle the columns of acidulated alcohol in the burette and the control tube can be raised and lowered. The burette and the control tube are enclosed in part in a common water jacket to preserve an equal temperature in both. The control tube has attached to it a movable marker (x) for recording the height of the fluid column. At its top, it opens to the air through a tap which is usually kept closed, and at all events is never opened during the course of any single test.

The burette opens at the top in two ways: one through a stopcock to the exterior, the other leads by glass tubing to the absorption chambers. Through the stopcock the sample is taken into the burette for analysis. The chambers for absorption are made independent of

each other, of the burette, and of the exterior, by taps. The chamber to be used for O_2 absorption is filled with glass rods to increase the absorbing surface.

The instructor in charge will demonstrate the use of the apparatus before the experiments begin. The analyst should remember that tight connections, well-greased stopcocks and complete absorption of the gas are essential for accurate determinations.

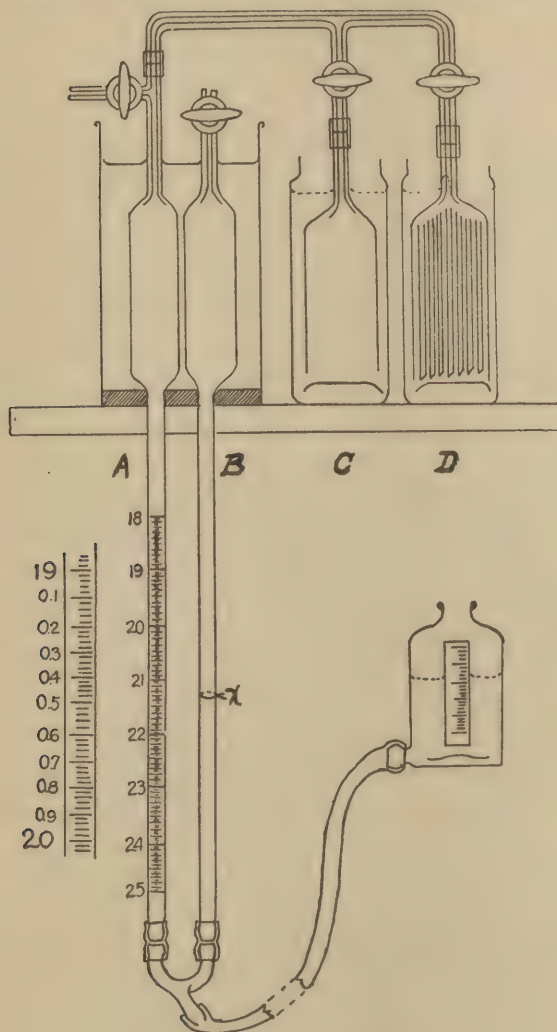


FIG. 10. — The Gas Analyzer

What is the purpose of the temperature-pressure tube?

Why is it necessary to wait for some time after absorption?

Preparation of the Analyzer.—With the fluid columns in the burette and the control tube level at about 23 c.c., set the marker at the lowest point of the meniscus in the control tube and close the tap at the top. Close the taps shutting off the hydroxide and pyrogallate chambers. Open the stopcock to the exterior; raise the leveling-bottle until the acidulated alcohol nearly reaches the side tube. Close the stopcock. Keeping the fluid levels equal in the leveling-bottle and the burette, open the tap to the hydroxide chamber and cautiously lower the fluid column in the burette, thus raising the height of the hydroxide column within the chamber. Bring the hydroxide column slowly to the scratch in the tubing a few centimetres below the tap, judging all fluid columns by the lowest point of the meniscus. When this is accomplished close the stopcock of the chamber of hydroxide, and proceed in a similar manner to bring the pyrogallate to its mark just below its tap.

Before taking a sample of gas into the analyzer for analysis the connecting tubes between the stopcock and the absorption chambers *must be freed from the gas or gases*

to be measured. Open the stopcock to the exterior and lower the meniscus in the burette to a level between 23 and 25 c.c. The pyrogallate solution is alkaline and absorbs both CO_2 and O_2 . Slightly raise the column in the burette by raising the leveling bottle. Open the tap to the pyrogallate chamber. The column of the absorbent will fall. Continue raising the leveling bottle until the fluid nearly fills the enlargement of the burette, but take care that no gas escapes through the absorbing bottle. Now cautiously lower the leveling bottle and draw the gas back into the burette until the solution rises again to the top of the chamber. Since some of the gas has been absorbed, the fluid column cannot be returned to its original level without drawing the reagent over. Therefore *exercise great care to avoid*

drawing the absorbent as far up as the stopcock. Should that occur, the apparatus must be cleaned by removing and thoroughly washing the stopcock, and by passing acidulated water through the tube.

When the absorbent has been brought up to a convenient height, not necessarily to the mark below the tap, lift the leveling bottle, send the gas back into the absorbing chamber again, and then with caution withdraw it as before. Before reading the burette, wash the gas in the absorbent in this manner twelve times. At the close of the twelfth washing, bring the height of the pyrogallate column to the mark below its tap as at the beginning of the experiment, and shut off the tap.

In making a reading after absorption of part of a sample, a correction must be made for changes in the temperature or atmospheric pressure in the course of the analysis. Proceed as follows: Before reading the burette, wait three or four minutes, *meanwhile agitating the water bath* by blowing through the immersed tube. Bring the column in the control tube to the level of the marker. Measure the difference between the fluid level in the tube and the fluid level in the bottle by means of the millimetre scale on its side. Bring the leveling bottle into such a position that its meniscus is a corresponding distance above or below that in the graduated burette and then read the volume of the remnant of the sample. This is the original volume of the gas less some of the O_2 .

Before the analyzer is ready, it is necessary to make a second check absorption to determine if all the O_2 has been taken out. This is done in exactly the same manner as before. The manipulation is complete if the second reading agrees with the first: if it does not, more readings must be taken, until two minimal readings agree. The analyzer is now ready for use.

Determination of Atmospheric Oxygen.—Open the stopcock to the exterior and expel all the gas in the burette until the acidulated alcohol drips from the side tube. By slowly and continuously lowering the leveling bottle take in from 23 to 25 c.c. of the room air. Close the stopcock. This is the sample of air for O_2 analysis.

Open the tap at the top of the control tube and bring the meniscus in this tube to the same level as that in the burette. Wait three or four minutes, meanwhile agitating the water in the jacket. Now, with the two fluid levels even, set the marker at the meniscus in the control tube and close the tap at the top. Read and record the volume of the sample taken for analysis.

Proceed with the absorption of the O_2 in this sample (the amount of CO_2 is negligible) in exactly the same manner as before, and continue until two minimal readings agree. Your data for submission should then appear somewhat as follows:

(a) Volume of Air	24.20 c.c.
(b) Volume of Air — O_2	
(1)	19.15 c.c.
(2)	19.14 c.c.
(3)	19.14 c.c.
(c) Minimal Volume — O_2	19.14 c.c.
(d) Volume of O_2 (= the original volume of the sample minus the volume remaining after absorption).	5.06 c.c.
(e) Percentage of O_2 (= the volume of oxygen divided by the volume of the sample, the quotient $\times 100$)	20.91 %.

Summary

The process of analysis after preparation of the analyzer may be summarized for convenience as follows. This procedure *must be followed rigorously*, as no unnecessary manipulation has been included. *No variations are permitted*, except upon the advice of the instructor in charge.

1. Make sure that the solvents in the absorbing chambers are at their proper levels.
2. Inspect all stopcocks to ascertain if each is properly adjusted.
3. Expel all gas from the burette.
4. Admit a convenient amount of the sample.
5. Bring the fluid level in the temperature-pressure tube to a height equal to that in the burette.
6. Wait three or four minutes, stirring the water in the jacket.
7. Mark the meniscus in the temperature-pressure tube when its gas content is at atmospheric pressure, and close the tap.
8. Record the volume in the burette.
9. Send the gas into the pyrogallate chamber twelve times.
10. Adjust the height of the column in the chamber.
11. Lower the leveling bottle until the column in the temperature-pressure tube is brought to its mark as at the beginning, and measure the pressure change.
12. Read the corrected volume in the burette and record.
13. Repeat 9 to 12 inclusive, read and record; continue until the readings are constant.
14. Make the calculations, and report the result to the instructor.

After expelling the gas now in the burette until the water drips from the side tube, the apparatus is ready for another analysis.

Determination of CO_2 in Expired Air. — For this purpose a collector (consisting of rubber bag and mouth-piece), after having been completely emptied of air, and with the valve at the mouth-piece closed, is filled through the small side valve with 1000 c.c. of room-air from a graduated carboy. See that the analyzer is ready for use. The subject now securely occludes the nostrils with the nose clip, and, placing in his mouth the mouthpiece, with its rubber collar between his lips and teeth, he breathes through the valve the room-air. When ready and quiet, the experimenter turns the simple two-way valve a quarter turn so that the subject breathes the air in the bag. The subject continues rebreathing to the "breaking point," that is, until he can no longer breathe without extreme unpleasantness. Upon indicating this point to the experimenter by a pre-arranged signal, the latter turns the valve again so that the subject breathes room-air. The apparatus may now be removed. The bag contains air enriched with CO_2 .

Wash out the short rubber tube connected with the smaller valve of the collector by expelling through it a small amount of enriched air. Connect it at once to the entrance tube of the analyzer. When ready for analysis, open the smaller valve of the collector, open the stopcock of the analyzer, and take into the burette a convenient amount of the gas in the bag; 25 c.c. Go carefully through all the steps given in the summary of the analysis of atmospheric oxygen, only substituting in 9 the "hydroxide" for the "pyrogallate chamber." Make three determinations of the sample, and tabulate them as follows: —

DETERMINATION OF CO_2 IN EXPIRED AIR

(a) Volume of Sample.....	22.00 c.c.
(b) Volume of Sample — CO_2	
(1).....	20.48 c.c.
(2).....	20.45 c.c.
(3).....	20.45 c.c.
(c) Minimal Volume — CO_2	20.45 c.c.
(d) Volume of CO_2	1.55 c.c.
(e) Percentage of CO_2	7.04.

Report the result to the instructor.

What did you observe regarding the behavior of the subject as he approached the "breaking point" ?

To what were these effects due ?

What would have occurred had the rebreathing continued longer ?

The Basal Metabolism. — On the day before the experiment, one man from each group of four is to be selected to act as subject for this and the following experiment. For the metabolism determination he must report to the laboratory without breakfast, and must be able to give details concerning the time and content of his previous meal. On coming to the laboratory he prepares for the experiment. He first urinates, noting the exact time. The urine may be discarded. He then assumes a comfortable reclining position near the spirometer and rests completely relaxed for a half-hour before the observation is begun. Five minutes before the collection of the expired air is started, adjust in his mouth the mouthpiece of the spirometer (Henderson, *J. Biol. Chem.*, 1918, xxxiii, p. 48) and put on the nose clip. First wash out the spirometer by means of the subject's expired air. To do this the subject breathes into the spirometer until it is partially full: the bell is then forced down with the small valve in the cone open for escape of the air: the process is repeated once or twice in order to fill the dead space under the cone with expired air, and the valve is closed. Once this operation is completed, no repetition is necessary during the experiment. Now with the spirometer empty except for the dead space, and its bell forced to its lowest point, turn the spirometer valve to the room air. Meanwhile one operator must prepare the gas analyzer for an analysis, while another makes ready a kymograph with the appropriate attachments for recording the respiration. Prepare also the form for recording the various determinations as given at the end of this experiment.

Record the bell's lowest height as read on the attached scale. Make sure that the thermometer is properly placed. During the experimental period, no movement whatever of the subject is permissible: he must lie wholly at repose: he may not talk or attempt to direct the operations. The accurate determination of basal metabolism depends upon the attainment of a minimum of functional activity in the subject. On the other hand, he may not sleep. The conditions of basal metabolism ("standard metabolism," Krogh) are defined as those obtaining in wakefulness with elimination so far as possible of all voluntary muscular movements and with no food being digested or absorbed.

When these preparations are complete, the first period of ten minutes, by watch, may be run. Simultaneously, at a signal from one of the operators, the valve of the spirometer is turned so that the subject exhales into the bell, and the kymograph is started at a slow speed to record respiration. An assistant counts the pulse and reports it minute by minute. At the close of the ten-minute interval, as observed by the second hand of an accurate watch, the valve of the spirometer is turned again to the room air, and the kymograph stopped. As the respiration rate is significant in the computations, the kymograph must be started and stopped promptly. No time-recording device is necessary. This is Period I. (The capacity of the spirometer may not permit a ten-minute period. In that case a six- or seven-minute period must suffice. A mark on the scale shows when the bell has risen to a point 5 cm. from the limit. Stop the collection at the end of the first minute after this point has been passed.)

Three periods are to be taken. During the intervals between them, the subject is not to be disturbed, but the mouthpiece of the spirometer may be taken out to rest him. The pneumograph should not be removed. Immediately after each period proceed quickly, but carefully, as follows: —

1. Read the height of the spirometer bell, and record.
2. From the thermometer in the bell, record the temperature of the expired air.
3. Record the barometric pressure and the room temperature.
4. Record the time and the duration of the period.
5. Designate the number of the period over the respiration record on the kymograph.
6. Obtain by use of the two sampling bottles some of the expired air from the bell of the spirometer. For a good sample of the well-mixed gas, connect to the small valve of the spirometer the tube of one of the bottles, which has been completely filled with the acidulated water, by raising the other bottle. Lower the other bottle, thus drawing in some of the gas: send it back into the spirometer and withdraw it several times. After the final withdrawal close the valve of the bell, screw tight the clip on the tube, and remove the bottles. A large enough sample for two or three check analyses should be obtained.
7. Designate the number of the period on the sampling bottle in case the analysis is not to be made immediately. Keep the water level in this bottle lower than in the other in order to prevent a possible leak of room air into the sample.

The remaining two periods are to be conducted in exactly the same manner as above outlined. At the close of the experiment the subject should urinate into a clean bottle and the time should be noted on the bottle together with the time of the previous urination. This urine will be preserved and the *total nitrogen* determined by one of the operators, using the method advised in biological chemistry. From this, calculate the number of grams of protein metabolized per hour, one gram of urinary nitrogen representing 6.25 grams of metabolized protein.

As soon as possible after collecting them, analyze each of the samples of gas, first for CO_2 , then for O_2 , until two analyses check within the experimental error of the analyzer. (The analysis for O_2 must not be made first, because the pyrogallate solution is alkaline and will absorb CO_2 .) Meantime, the name, age, stripped height in centimetres, and stripped weight in kilograms of the subject are recorded on page 146. The stripped weight is estimated for these purposes with sufficient accuracy by deducting 4.5 kg. from the gross weight, and the stripped height by the subtraction of 2.5 cm. from the gross height.

Summary

1. After he has voided, keep the subject at repose for the required rest period.
2. Adjust the apparatus and prepare the gas analyzer.
3. Fill the dead space of the spirometer with mixed expired air.
4. Record the lowest height of the spirometer bell when empty.
5. Turn on the spirometer, and simultaneously start the kymograph.
6. Run a ten minute experimental period, or such part thereof as is possible.
7. Turn off the spirometer, and simultaneously stop the kymograph.
8. Read and record the height of the spirometer.
9. Record the temperature of the exhaled gas.
10. Record the barometric pressure and the room temperature.
11. Record the time and duration of the period.
12. Obtain a sample of the gas in the spirometer.
13. Prepare the kymograph for the next period.
14. Expel the air from the spirometer.
15. Run the succeeding periods, by steps to 4 to 12 inclusive.
16. Analyze the exhaled gas.
17. Take the measurements of the subject.
18. Make the computations and record them.
19. Have the subject void into a carefully labelled bottle and determine the urinary nitrogen excretion per hour.
20. Report the results to the instructor.

Definitions and Computations. — The *surface area* is obtained from the height and weight of the subject by reference to the curves by DuBois (Arch. Int. Med., 1916, xvii, pp. 862–871) reproduced in Fig. 11. These curves were plotted from the equation,

$$S = W^{0.425} \times H^{0.725} \times 71.84$$

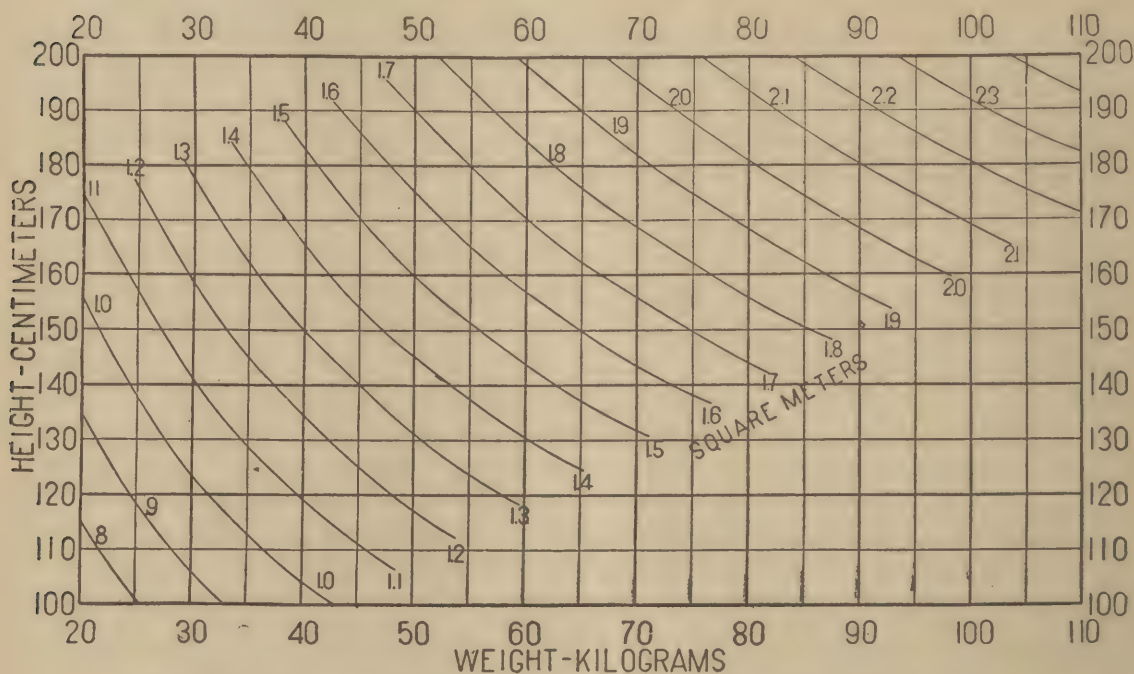


FIG. 11. Chart for determining surface area (metres) from weight (kilograms) and height (centimetres).

where S is surface area in square metres, W is weight in kilograms, H is height in centimetres, and 71.84 is a constant for man.

The *respiration rate* per minute is determined from the kymograph record by dividing the total number of respirations by the length of the period in minutes.

The *total volume of expired air* is calculated by multiplying the distance through which the spirometer bell has risen by the area of cross-section. The area of a circle is $3.1416 \times r^2$, and the radius of the bell is stated on a card attached to the spirometer.

The volume thus measured must be corrected to give the volume of dry air at standard temperature (0°C.) and pressure (760 mm. Hg). In making this correction, an error in the barometer and the moisture of the expired air must be considered: —

(1) The barometer tube lengthens with heat and shortens with cold, and the barometric readings must be corrected for this change. In the following table¹ are found the temperature corrections for reducing to 0°C. the readings of a mercury barometer: —

Temp.	700 mm.	710 mm.	720 mm.	730 mm.	740 mm.	750 mm.	760 mm.	770 mm.
15°	1.69	1.72	1.74	1.77	1.79	1.81	1.84	1.86
20°	2.26	2.22	2.32	2.36	2.39	2.42	2.45	2.48
25°	2.83	2.87	2.91	2.95	2.99	3.03	3.07	3.11

¹ This and following tables were taken from Pearce: Jour. Lab. and Clin. Med., 1918, iii, p. 420.

Subtract from the height of the barometer the appropriate quantity as found in the table. The table is for a barometer with a brass scale; the values are a little lower (about .2 mm.) than for the glass scale. The corrections for intermediate temperatures can be approximated.

(2) The expired air is saturated with moisture. To learn the true pressure of the dry air, the pressure due to water vapor must be subtracted from the corrected barometric pressure. The vapor tension of water at various temperatures is presented in the following table: —

Temp.	15°	16°	17°	18°	19°	20°	21°	22°	23°	24°	25°
Mm. Hg.	12.7	13.6	14.4	15.4	16.4	17.4	18.5	19.7	20.9	22.2	23.5

To obtain the dry barometric pressure, subtract from the corrected barometric pressure at the time of the experiment the mm. Hg. corresponding to the temperature of the expired air.

The coefficient of expansion of gases is taken as 0.003665, or $1/273$; therefore, the volume at 0° equals the volume at 1° divided by $1 + 0.003665 t$; and hence

$$V_0 = \frac{V \times 273}{273 + t} = \frac{V}{1 + 0.003665 t},$$

when V_0 = Volume at 0° and V = Volume at t° . The volume of gas being inversely as the pressure, $V_0 = \frac{VP}{760}$, where V equals volume at P pressure; or working both corrections together,

$$V_0 = \frac{VP \times 273}{760 \times (273 + t)} = \frac{VP}{760 (1 + 0.003665 t)}.$$

The calculation may be abbreviated by reference to the following table of factors for reducing gaseous volumes to standard temperature and pressure: —

Mm.	15°	16°	17°	18°	19°	20°	21°	22°	23°	24°	25°
720	.898	.894	.891	.888	.885	.882	.880	.877	.873	.870	.867
730	.910	.907	.904	.901	.897	.894	.891	.888	.885	.882	.879
740	.922	.919	.916	.913	.910	.907	.904	.901	.897	.894	.891
750	.935	.932	.928	.925	.922	.919	.916	.913	.910	.907	.904
760	.947	.944	.941	.938	.934	.931	.928	.925	.922	.919	.916
770	.960	.957	.953	.950	.948	.945	.940	.936	.933	.930	.927

The observed volume, when multiplied by the factor corresponding to the temperature and *corrected pressure*, will give the volume of the expired air reduced to 0° and 760 mm.

The total volume exhaled during the period, divided by the time in minutes gives the *ventilation per minute*, or the *minute volume*.

The minute volume divided by the respirations per minute gives the *tidal volume*, or the *volume per respiration*.

To obtain the net percentage of CO_2 produced by the organism during the experimental period, it is necessary to deduct from the percentage of expired CO_2 in the spirometer the percentage of CO_2 in the inspired air: 0.03%. The total metabolized CO_2 in the spirometer at the close of the period

is found by multiplying the total volume of the expired air by this corrected percentage. The volume of CO_2 eliminated by the organism per minute is, therefore, calculated as follows: —

$$\frac{(\% \text{CO}_2 \text{ in sample} - \% \text{atmospheric CO}_2) \times \text{total vol.}}{\text{Time in minutes}}$$

To learn the O_2 consumption, the O_2 percentage found by analysis must likewise be corrected because O_2 has been taken up more than CO_2 has been given off in the process of respiration, and the volume of dry air has consequently diminished. Since N_2 is passive in the respiratory process, it is possible to compute from the N_2 percentage in the expired air, on the basis of the constant composition of the atmosphere, the amount of O_2 inspired with it. The ratio of O_2 to N_2 in atmospheric air is as 20.93 to 79.04, or as 0.2649 to 1. By multiplying the percentage nitrogen found in the expired air by the constant factor, 0.2649, there is found the corresponding volume of O_2 . The whole computation may be summarized thus: —

Percentage N_2 in expired air = 100 — (percentage O_2 + CO_2).

Volume O_2 corresponding to N_2 present (*i.e.*, O_2 inspired) = percentage N_2 in expired air \times 0.2649.

The N_2 percentage in expired air is dependent on the amount of CO_2 and O_2 in it. In the following table is given the volume percentage of O_2 which would be present in the inspired air to account for the CO_2 + O_2 found in the expired air.

% CO_2 + % O_2 In Expired Air	Vol. % of O_2 In Inspired Air	% CO_2 + % O_2 In Expired Air	Vol. % of O_2 In Inspired Air
19.4	21.38	20.4	21.10
19.5	21.35	20.5	21.07
19.6	21.31	20.6	21.04
19.7	21.28	20.7	21.01
19.8	21.25	20.8	20.98
19.9	21.22	20.9	20.96
20.0	21.20	21.0	20.93
20.1	21.18	21.1	20.90
20.2	21.15	21.2	20.88
20.3	21.13	21.3	20.86

The actual % O_2 in the expired air is known from the direct analysis of the sample; hence the percentage O_2 consumed is revealed by the following subtraction:

Percentage O_2 in inspired air — percentage O_2 in expired air. This multiplied by the minute volume yields the O_2 consumption per minute.

The *respiratory quotient* is found by dividing the CO_2 production per minute by the O_2 consumption per minute. Calculate it. The physiological significance of this factor will be treated in detail in the conference and in the lectures.

Find the *total calories produced per hour*. From the CO_2 and O_2 consumption per minute calculate the litres of these gases exchanged per hour. When the urinary nitrogen has been determined, continue the calculation as illustrated below. The figures to be used are:

1 gram of urinary nitrogen represents in protein metabolism 6.25 grams of protein, and 26.5 calories, and in the respiratory exchange 4.76 litres of CO_2 or 9.35 grams, and 5.91 litres of O_2 or 8.45 grams.

By deducting the protein portion from the gaseous exchange and then solving the proportion of the two remaining foodstuffs by means of Table 2, estimate the proportion and actual amount of the foodstuffs burned. The calculation may be illustrated thus:—

	Litres CO ₂	Litres O ₂
Original respiratory exchange per hour	12.72	16.21
Deduct that due to protein	2.38 (Ur. N per hour × 4.76)	2.96 (Ur. N × 5.91)
Non-protein in respiratory exchange	10.34	13.25
Non-protein respiratory quotient (R.Q.)	$\frac{\text{Litres CO}_2}{\text{Litres O}_2} = \frac{10.34}{13.25} = 0.780$	

From the R.Q. and the non-protein O₂ find in Table 2 the total non-protein calories = 63.28 cal.

Percentage in calories of the non-protein exchange of carbohydrates = 25.2 %; of fat = 74.8 %.

Calculate from this the calories used per hour, of carbohydrate = 15.9 cal.; of fat = 47.3 cal.

Determine the total caloric output per hour, as shown by the consumption of *all three* foodstuffs.

TABLE 2

THE SIGNIFICANCE OF THE NON-PROTEIN RESPIRATORY QUOTIENT AS REGARDS THE HEAT VALUE OF 1 LITER OF OXYGEN, AND THE RELATIVE QUANTITY IN CALORIES OF CARBOHYDRATE AND FAT CONSUMED

R.Q.	Calories for 1 Liter of O ₂ Number	Carbohydrate Per cent	Fat Per cent
0.70	4.686	0.0	100.0
0.71	4.690	1.4	98.6
0.72	4.702	4.8	95.2
0.73	4.714	8.2	91.8
0.74	4.727	11.6	88.4
0.75	4.739	15.0	85.0
0.76	4.752	18.4	81.6
0.77	4.764	21.8	78.2
0.78	4.776	25.2	74.8
0.79	4.789	28.6	71.4
0.80	4.801	32.0	68.0
0.81	4.813	35.4	64.6
0.82	4.825	38.8	61.2
0.83	4.838	42.2	57.8
0.84	4.850	45.6	54.4
0.85	4.863	49.0	51.0
0.86	4.875	52.4	47.6
0.87	4.887	55.8	44.2
0.88	4.900	59.2	40.8
0.89	4.912	62.6	37.4
0.90	4.924	66.0	34.0
0.91	4.936	69.4	30.6
0.92	4.948	72.8	27.2
0.93	4.960	76.2	23.8
0.94	4.973	79.6	20.4
0.95	4.985	83.0	17.0
0.96	4.997	86.4	13.6
0.97	5.010	89.8	10.2
0.98	5.022	93.2	6.8
0.99	5.034	96.6	3.4
1.00	5.047	100.0	0.0

Divide the total calories per hour by the square metres of body surface to determine the *calories per hour per square metre*.

Basal Metabolism is defined as the average production of calories per hour per square meter of surface area. Find the normal metabolism of the subject from Table 3; and calculate his *percentage normal metabolism*.

TABLE 3
STANDARDS OF NORMAL BASAL METABOLISM
Av. Cals. per Hr. per sq. metre of body surface

Age., Yrs.	Men	Women
8-9 inc.	54.0	54.0
10-11.	51.5	50.0
12-13.	50.0	46.5
14-15.	46.0	43.0
16-17.	43.0	40.0
18-19.	41.0	38.0
20-29.	39.5	37.0
30-39.	39.5	36.5
40-49.	38.5	36.0
50-59.	37.5	35.0
60-69.	36.5	34.0
70-80.	35.5	33.0

Using the figures, 1 gr. carbohydrate = 4.1 cal., 1 gr. fat = 9.3 cal., and 1 gr. protein = 4.1 cal., calculate the consumption of carbohydrate, fat and protein per hour under the conditions of the experiment.

Why is complete repose and prohibition of food essential for a metabolism determination ?

How would food and activity affect the respiratory quotient ? Why ?

Respiratory Exchange and Metabolism in Work. — With the same subject as in the preceding exercise again reporting at the laboratory without breakfast, conduct three periods while he is on the stationary bicycle instead of in a reclining position. Seat the subject on the bicycle, attach the pneumograph, and place the mouthpiece of the spirometer in his mouth with the valve open to room air. Now let him begin exercising vigorously. After two minutes, and while the vigorous exercise is continuing, at a given, pre-arranged signal, start the kymograph, turn the valve for expiration into the spirometer, and start collecting expired air. At the end of three minutes signal the subject to cease work, and if the bell is not yet filled, finish the collection of air with the subject at rest on the bicycle. Complete three periods with the same calculations and precautions used in the previous experiment. The subject is to urinate before and after the experiment, noting the exact time, and the last urine is to be carefully saved and analyzed for total nitrogen.

Metabolism after Eating. — Allow the subject to get a meal, preferably of carbohydrates, and determine the respiratory quotient and the metabolism about two hours after the meal.

Explain physiologically the variations in the respiratory quotient and metabolism introduced by work and by eating.

Why is a carbohydrate diet preferable to test the effects on the metabolism of eating ?

From what source is the extra energy derived which is utilized at first in exercise ?

What does this suggest in relation to diet ?

Determination of Basal Metabolism by Measurement of Oxygen Absorption. — The foregoing experiments have revealed the principles underlying the estimation of the metabolic rate. Since there is immediate dependence of the body on the supply of oxygen, and since

the respiratory quotient of persons in the post-absorptive condition or standard basal condition is approximately .82, knowledge of the amount of oxygen absorbed permits calculation of the amount of carbon dioxide given off, and thereafter all the further calculations which have been made in working out the inferences and conclusions in connection with direct analysis of the respiratory gases. A simple apparatus which allows accurate measurement of the use of oxygen by the body in a given time has been devised by Benedict. (Benedict and Benedict, *Boston Med. & Surg. J.*, 1923, clxxxviii, p. 567.) As shown in Fig. 12 the apparatus consists essentially of three parts: a reagent can with a flexible rubber top, tube connections through valves and a mouthpiece; and a pump to supply quantitatively oxygen or air to the closed system. In principle the subject breathes air in from the can through

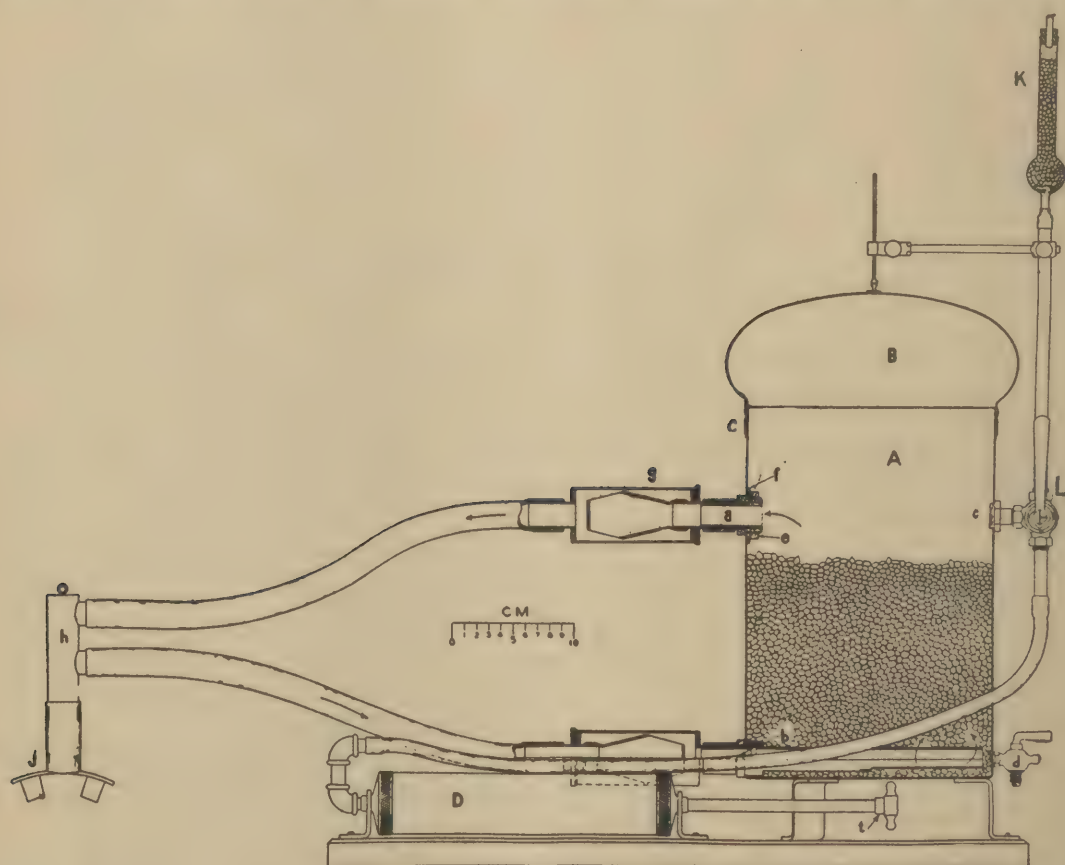


FIG. 12. — THE BENEDICT STUDENT FORM OF RESPIRATION APPARATUS

A, metal can partly filled with soda-lime; B, rubber bathing cap, held in place by rubber band, C. The subject breathes through the mouthpiece, *j*, which is attached to the metallic piece, *h*, connecting with two rubber tubes leading to Saddle respiratory valves on the can, A. The expired air enters through the opening, *b*, and is returned to the subject, freed of carbon dioxide, through the opening, *a*. Oxygen for preliminary enrichment is admitted through the petcock, *d*, and measured amounts of dry air, drawn through the calcium-chloride tube, K, into the pump, D, are forced into the can through the valve, L, and the opening, *e*.

one valve and passes the expired air back through another valve into the can in which the CO₂ is removed by soda-lime. The flexible rubber top (a bathing cap) allows large changes in volume with each respiration, as well as a gradual decrease in volume, due to the absorption of O₂ by the subject. The amount of O₂ disappearing thus in unit time is the measure desired.

With all the conditions for basal metabolism provided — absence of digestion, a preliminary period of rest, complete muscular repose, and normal temperature — conduct the determination as follows:—

Insert the mouthpiece between the teeth and the lips.

Press the rubber top into the can as far as possible and let in O_2 through the petcock at the bottom until the button at the top of the bag rises slightly above the bottom of the vertical rod held above it. Thus the air in the can is sufficiently enriched with O_2 to prevent a condition of oxygen-want during the period of the experiment, although the later additions to the bag are room air.

At the end of normal expiration, as judged by the rise and fall of the chest of the subject, attach the nose clip.

The bag will now rise and fall with each respiration. At the end of each expiration the button will press less and less against the rod, and shortly it will just touch. At this point the exact time should be noted on a watch, and first the position of the second hand, then the position of the minute hand and the hour carefully recorded.

Turn the valve, L, so as to connect the pump with the calcium-chloride tube, K, and draw the pump handle completely out. Now turn the valve to connect the pump with the can and slowly push the dry air into the can. Make any major movement of the plunger when the bag is high, *i. e.*, towards the end of expiration, and thus avoid introducing an excessive amount of air.

As the plunger nears the end of a complete stroke drive all the air out of the barrel so that a slight excess is present in the rubber bag, *i. e.*, the button rises slightly above the lower end of the vertical rod. Now wait, as at the start, until at the end of an expiration the button just touches the rod. Again record the time in seconds and minutes. The interval represents the period required for absorption of the oxygen in one complete pump stroke. Repeat the procedure for six full strokes, keeping careful record of the elapsed time after the completion of each stroke.

At the end of the sixth stroke remove the nose clip and mouthpiece, record the temperature of the pump and the barometer. The temperature of the pump is obtained by laying a laboratory thermometer with its bulb in contact with the brass barrel. During the determination *do not place the hand on the barrel of the pump*. Why?

The capacity of the pump is stated on each instrument. The tension of aqueous vapor does not enter into calculation, because the air drawn into the pump is dried by passing through tube K, and the air over the soda lime is essentially dry. Since the only constituent of the air which has disappeared is oxygen, the volume of six pump strokes, reduced to standard pressure and temperature, represents the oxygen consumption during the period. Knowing the time required for this consumption, the oxygen usage per minute or per hour can be readily calculated.

If the total elapsed time at the end of each stroke is plotted on coördinate paper, with the time as the ordinates and the number of pump strokes as the abscissae, the accuracy of the final observation may be judged. If the apparatus has been well used, all seven points (including the zero) will fall in a straight line, which may be taken as the slope of the oxygen absorption. If the last reading has been aberrant, the oxygen consumption can be computed from the slope of the line.

Calculate the number of calories per square metre per hour produced by the subject, on the assumption that his respiratory quotient is .82. Even if the respiratory quotient is not .82, the error is not great. The probable limits of the quotient are .73 and .95. The calorific value of O_2 at .73 is 4.714 calories per litre and at .95 is 4.985 calories. The difference, .271, makes the maximum variation either side of the calorific value for the average quotient of .84 amount to 2.8 per cent.

Name..... Date.....
 Age..... Physical Condition.....
 Height (cm.)..... Time of last previous meal.....
 Weight (kg.)..... Contents.....
 Surface Area (sq. m.)..... Carbohydrate (approx. gms.).....
 Fat (approx. gms.).....
 Protein (approx. gms.).....

	At rest				At work			
	I	II	III	Av.	I	II	III	Av.
Period No.								
Hour.....								
Duration.....								
Pulse rate per min.								
Respiration rate per min.								
Minute Volume (Standard Condition), c.c.								
Tidal Volume (Standard Condition), c.c.								
CO ₂ per min., c.c.								
O ₂ per min., c.c.								
Respiratory Quotient.....								
Total Calories per hour.....								
Calories per hour per sq. m.								
Percent, Normal Basal Metabolism.....								
Calories derived per hour from:								
Carbohydrate.....								
Fat.....								
Protein.....								
Metabolism of foodstuff in gms. per hr.:								
Carbohydrate.....								
Fat.....								
Protein.....								

RESPIRATION

AMONG the functions of the blood and lymph, already noted, were those of conveying from the lungs to the tissues the oxygen required for oxidation, and from the tissues to the lungs the carbon dioxide resulting from oxidation. In order that fresh supplies of oxygen may be brought into the lungs and that the carbon dioxide eliminated into the alveolar spaces may be removed, it is necessary that the lungs be ventilated. Respiration, like digestion, may therefore be considered in its two aspects, in its chemical aspect, the oxidative processes in the active tissues: and in its physical or mechanical aspect, the ventilation of the lungs.

The Mechanics of Respiration

Observation of the Thoracic and Abdominal Walls.—Let one member of each pair of students, preferably the more slender, strip to the waist and sit with arms and hands symmetrical and with both sides of the chest evenly illuminated.

While the subject is breathing quietly, observe whether the two sides of the chest move equally. What change occurs in the antero-posterior diameter of the chest?

In the lateral diameter?

Is there alteration in the intercostal spaces?

By means of a tape, measure the amount of chest expansion at the level of the axilla and at the level of the xiphoid cartilage. With calipers measure the changes in the lateral and antero-posterior diameters of the chest at these two levels. At which level is the enlargement greater?

Study the movement of the ribs during inspiration and expiration. By what arrangement does their movement cause an enlargement of the thorax in two directions?

What are the movements of the abdominal wall during respiration?

How are these movements correlated with the action of the diaphragm?

Observe the muscles concerned in quiet inspiration and expiration, and enumerate them.

Repeat all these tests and observations with forced inspiration and forced expiration. What additional muscles are now used?

***Respiratory Pressure in Man.**—To a mercury manometer connect a rubber tube with a mouthpiece. Hold the mouthpiece tightly between the lips, and observe, in millimetres of mercury, the air pressure, positive or negative, during quiet normal inspiration and expiration through the nose; similarly, but with the nostrils closed, during forced inspiration and expiration.

Record of Human Respiration; the Pneumograph.—Arrange a recording tambour to write on a smoked drum. Connect the tambour to a Fitz pneumograph by means of a rubber tube provided with a side branch. Arrange an electromagnetic signal attached through a simple key to a dry cell. Fasten the pneumograph about the chest. Close the side branch of the connecting tube, and let the recording begin. The subject should not

see the curve as it is being written. At regular intervals (5 or 10 seconds) make a contact with the simple key and thus write a time record below the curves of respiration. How rapid is the respiration per minute? Compare the inspiratory and expiratory phases in duration. (Fitz, J. Exp. Med., 1896, i, 677.)

Secure a respiratory tracing during a period of laughing, during a period of coughing, and while reading aloud. What is the characteristic effect of these actions on respiration?

The Volume of Respired Air. — The amount of air that can be forcibly expired from the lungs after normal quiet expiration is complete is known as *supplemental air*. The amount that can be voluntarily inspired after normal inspiration is complete is called *complemental air*. The amount of air breathed in and out in quiet respiration is the *tidal air*. These three amounts taken together — the total quantity that can be expired after inspiring as much as possible — equal the *vital capacity*.

By means of a spirometer determine the vital capacity. Determine the volume of supplemental, and of supplemental plus tidal air. Calculate the tidal and complemental air. Compare the result with a determination of the tidal air by means of the spirometer — a normal expiration after a normal inspiration. Make several determinations and take the average in each instance. Compare the vital capacity in the prone and erect postures.

Hand to the instructor the results of these observations, together with the measurements of the girth of the chest at the axillary level and at the level of the xiphoid cartilage in forced inspiration and in forced expiration. Also state the usual exercise and sports, and whether previous residence has been in a hilly or level country. These data will be considered with the class in a discussion of the process of evaluation of such data.

Auscultation of Respiratory Sounds. — During respiration characteristic sounds can be heard over the chest. These sounds are modified in pathological conditions of the lungs. It is important for the medical student to become acquainted with the normal sounds, as a basis for judgment of the abnormal. There are two classes of sounds. (Laennec, *De l'auscultation ou traité du diagnostic des maladies des poumons et du cœur*, Paris, 1819; Skoda, *Abhandlung über Percussion und Auscultation*, Wien, 1839.)

Bronchial Breathing. — With a stethoscope listen over the larynx or trachea during quiet respiration. A loud aspirating sound is heard corresponding closely to the sound produced by breathing through the mouth held in a position to pronounce *h* or *ch*. This is the sound of bronchial breathing. Is the sound heard during both inspiration and expiration? Place the stethoscope successively at points more and more remote from the glottis, over the course of the trachea and bronchi, and note how deeply in the lungs bronchial breathing can be heard. Does strong rapid respiration increase the area over which this sound is audible?

Vesicular Breathing. — Listen with the stethoscope near the fifth right intercostal space during quiet respiration. A gentle *soughing* sound will be heard, resembling that made by breathing through the lips when they are in a position to pronounce *f*. This is the vesicular breathing sound.

After hearing this sound distinctly, determine whether it can be heard during expiration as well as during inspiration. At what time during inspiration is it loudest? If the vesic-

ular sound is not clearly distinguished from the bronchial sound, listen with the ear directly against the chest wall (direct auscultation).

Let the subject respire forcibly. How is the character of the vesicular breathing now altered?

Examine the chest sounds systematically and symmetrically first on the one side and then on the other. Note any characteristic differences in the sounds, either in intensity or duration.

Palpation of the Chest during Phonation; Vocal Fremitus. — While the subject repeats "one, two, three," or pronounces continuously the letter *r*, apply the tips of the fingers or the palm of one hand alternately to symmetrical points on the chest wall. The vibrations of the vocal cords not only cause the external air to vibrate, but also the air in the trachea and bronchial tubes. These vibrations traverse the lung tissue and the chest wall and may then be felt by the palpating hand.

Auscultation may be used as well as palpation in studying vocal fremitus. An accumulation of fluid in the pleural cavity reduces or suppresses vocal fremitus. Why?

The fremitus is increased over a portion of lung solidified by pneumonia. Why?

Percussion of the Chest. — A very valuable method of studying pathological changes in the chest is by means of the resonant note emitted by the chest when it is struck. It is necessary to have a clear understanding of the normal note and its variations and distribution over the chest, before judging the abnormal condition.

Lay the middle finger of the left hand between the first and second ribs on the right side, and with the middle finger of the right hand as a hammer percuss the area beneath. Now repeat the procedure at a symmetrical point of the left side. Examine the chest thus symmetrically, front and back. Areas will be found which are "dull," *i. e.*, give forth no percussion note. Outline the areas of dullness. Percuss the thigh or arm. Explain the areas of dullness discovered in examining the chest.

Analysis of Alveolar Air

The interchange between the blood and the air in the lungs occurs in the alveoli. In order to know the mode of the interchange it is necessary to learn first the partial pressures of the gases in the alveolar air. Between the nostrils and the depths of the lungs there is a gradual change in the gas mixture — the oxygen is less and the carbon dioxide more, the nearer the alveoli are approached. An amount of the inspired air, that varies under different conditions, fails to come into equilibrium with the gases in the blood passing through the pulmonary capillaries, before it is breathed out again. The amount that is thus breathed out determines the "dead space."

Method of Calculating the Dead Space. — It will be shown that

$$(1) \quad \%CO_2 \text{ in alveolar air} = \frac{\%CO_2 \text{ expired} \times \text{tidal air} - \%CO_2 \text{ inspired} \times \text{dead space}}{\text{Tidal air} - \text{dead space}}$$

Since inspired air (room air) contains negligible CO_2 , this becomes

$$(2) \quad \%CO_2 \text{ in alveolar air} = \frac{\%CO_2 \text{ expired} \times \text{tidal air}}{\text{Tidal air} - \text{dead space}}$$

Transposing this becomes:

$$(3) \text{ Dead space} = \text{tidal air} \left(1 - \frac{\% \text{CO}_2 \text{ expired}}{\% \text{CO}_2 \text{ alveolar}} \right)$$

The Collection of Alveolar Air (Haldane Method).—The gas analyzer is prepared for analysis, and a rubber tube about 2 cm. in diameter and about 1 m. long is attached near the mouthpiece to the intake stopcock. Close the nostrils with a clip. Breathe normally a few times; at the end of a *normal inspiration* put the mouth to the tube, breathe out quickly and fully, and close the mouthpiece with the tongue. Through an opening on the side of the tube the last air expelled from the lungs (which is alveolar air) is then drawn as quickly as possible into the burette (p. 132). This is the *inspiratory sample*.

Analysis of the Sample.—Proceed as in the analysis of atmospheric O_2 and air enriched with CO_2 (p. 135), only after process 14 in the summary has been completed for CO_2 and the volume recorded, begin at process 9, and absorb the O_2 in the same manner as before. Do not alter the height of the marker on the temperature-pressure tube, or open the stopcock to the exterior in the course of the analyses. The data obtained should be tabulated as follows:—

ANALYSIS OF ALVEOLAR AIR — INSPIRATORY SAMPLE

(a) Volume of Sample.....	23.00 c.c.
(b) Volume of Sample — CO_2	
(1).....	21.75 c.c.
(2).....	21.71 c.c.
(3).....	21.71 c.c.
(c) Minimal Volume — CO_2	21.71 c.c.
(d) Volume of CO_2	1.29 c.c.
(e) Percentage of CO_2	5.6
(f) Volume of Sample — CO_2 — O_2	
(1).....	19.38 c.c.
(2).....	19.38 c.c.
(3).....	19.38 c.c.
(g) Minimal Volume — CO_2 — O_2	19.38 c.c.
(h) Volume of O_2	2.33 c.c.
(i) Percentage of O_2	10.1.
Barometric pressure 762.2 mm. Hg.	

It was shown by Bert that the physiological action of gases depends not upon their percentage content, but upon their pressure. It is therefore necessary to calculate from the above data the partial pressure in the alveolar air of each of these gases for dry air. As the gas in the alveoli is saturated with water vapor at the body temperature, this factor is eliminated by subtraction from the barometric pressure, which will be posted daily on the blackboard. The aqueous tension of the vapour at 760 mm. Hg and 37°C . is 46.6 mm. For every mm. increase in atmospheric pressure, the aqueous tension decreases 0.061 mm. and vice versa. The calculation is thus made by the following formula:—

$$\text{p.p} = (\text{B} - \text{a.t.}) \times \% \text{ gas}$$

Where p.p = partial pressure of the gas in the alveoli, B = barometric pressure, a.t. = aqueous tension in the alveoli, % gas = percentage of CO_2 or O_2 . Thus the above analysis for CO_2 shows a partial pressure of that gas in the alveoli as follows:—

$$(762.2 - 46.4) \times 5.6/100 = 40.08 \text{ mm.}$$

For accuracy it is found that the results from the inspiratory sample must be averaged with the analyses of an *expiratory sample*. The procedure is much the same as for the inspiratory sample. Let the subject breathe normally for 5 minutes. When the analyzer is prepared and the experimenter is ready, collect a sample from a forced expiration made at the end of a *normal expiration*. The sample thus got is analyzed for CO_2 and O_2 , the pressure calculated, and the data tabulated as before. The average of the two samples gives the correct alveolar tension of CO_2 . Report the results to the instructor.

Alveolar Air in Work. — Make determinations of the alveolar CO_2 and O_2 on the subject after two minutes of exercise on the stationary bicycle.

Tabulate and calculate the results and report them to the instructor.

What is the effect of work on the partial pressure of the CO_2 in the alveoli?

What behavior did you observe in the respiratory activities of the subject after exercise? Account physiologically for these activities.

The Chemical Control of Respiration

Chemical changes in the blood have a marked effect upon the activity of the respiratory center. The amount of the different gases in the blood can be varied by variations of their content in the alveoli. The following experiments reveal the chief features of the chemical control of the respiratory mechanism.

The Effects of Breathing Room Air. — Let a subject whose alveolar air is known sit comfortably with a pneumograph recording his respirations. Prepare the spirometer as for metabolism measurements. After "washing out" the remnant of gas in the spirometer collect it nearly full of expired air, marking on the pneumograph tracing the times of beginning and ending the collection.

Measure (a) the volume of air expired per minute, (b) the per cent of carbon dioxide in the expired air, and (c) the number of respirations per minute.

Calculate (a) the volume of the tidal air (*i. e.*, the volume expired per respiration), and (b) the dead space, by equation (3) above, using the data already obtained for the alveolar air.

Compare the tidal volume as measured in this experiment with the volume of the tidal air as determined in the experiment on page 148.

The Effects of Breathing 5% Carbon Dioxide. — Pass enough carbon dioxide through the stopcock on the bell of the spirometer to raise the bell 2.5 cm.; close the cock and disconnect the tubing. Now draw in air through the cock until the marker is at 50 cm. Wait a few minutes for mixing, and then collect a sample for analysis. Reverse the mouthpiece so that the subject inspires the mixture in the spirometer. Since the first few breaths will contain room air from the tubing, neglect them and take the time for the bell to fall about 30 cm. Mark the pneumograph record to show the part recorded during this period. In a small bag, as demonstrated, collect a sample of the expired air during the latter part of the period.

Measure (a) the volume of air expired per minute, (b) the per cent of CO_2 in the expired air, and (c) the number of respirations per minute.

Calculate (a) the volume of the tidal air, and (b) the alveolar air, by equation (1) above.

The Effect of Breathing Increased Oxygen. — Repeat the foregoing procedure, but instead of introducing carbon dioxide into the spirometer let in 10 cm. of oxygen and then draw in room air to the 50 cm. mark. Is there any effect on the tidal volume and the number of respirations per minute produced by air enriched with oxygen?

The Effects of Anoxaemia. — Repeat the foregoing procedure, but first make a mixture in the spirometer of air two-thirds, nitrogen one-third. Measure and calculate as before. In addition to the carbon dioxide analysis determine the oxygen percentage and pressure in the inspired and expired air.

The Acute Effects of Oxygen Want. — Breathe to and fro through a can of soda-lime above which is attached a rubber bag containing air. Thus there is no accumulation of carbon dioxide, but a greatly diminished amount of oxygen. Observe on the pneumograph record whether the lessened oxygen supply has affected respiration. Stop the experiment as soon as unpleasant sensations of dizziness or faintness are experienced. Analyze the gas in the bag for its oxygen content.

The Effects of Decreased Carbon Dioxide. — By voluntarily forcing respiration the carbon dioxide content of the lungs and blood can be diminished.

Breathe deeply and more rapidly than is normal, but not faster than 30 times a minute. Stop the forced breathing when dizziness is felt. Secure a sample of alveolar air at this stage and analyze it. Is there a natural absence of respiration, *i. e.*, an apnoea?

Note how long the breath can be held after natural respiration, and compare it with the length of time the breath can be held after a minute of forced breathing.

How long can the breath be held after two or three deep inspirations of oxygen, following ordinary breathing. Compare this time with the time the breath can be held after a minute of forced breathing followed by the same inspirations of oxygen.

Compare the effects, on the rate of respiration, of running up stairs with and without previous forced breathing.

Tabulate the results of these experiments. What conclusions do you draw concerning the effects of oxygen and carbon dioxide in regulating the ventilation of the lungs?

The Nervous Control of Respiration

Besides chemical changes in the blood, nervous impulses play an important rôle in the control of respiratory activity. Conditions in different parts of the upper respiratory tract, the distension and collapse of the lungs, the act of swallowing, stimulation of afferent nerves in any part of the body, and also influences from the cerebrum can all affect profoundly the center governing respiration.

(Demonstration. — The Nervous Control of Respiration in the Rabbit. — A rabbit is anaesthetized with urethane (2 gms. per kg. by stomach). A small opening is made in the abdominal wall at the tip of the ensiform cartilage, the muscular slips of the diaphragm on the under surface of the cartilage are freed, and their tendinous ends connected with a recording lever. These slips, with normal blood and nerve supply, are representative of the whole diaphragm. Apply ammonia vapor to the nostrils and record the effect on respiration. Introduce a small catheter into the pharynx and larynx and stimulate the mucosa of the

region and again record the results. Stimulation of the superior laryngeal nerve may also be tried. Repeated inflation of the lungs (positive ventilation) results in an expiratory apnoea. Repeated deflation of the lungs, by rhythmic suction (negative ventilation), ends in an inspiratory apnoea. Cut one vagus nerve and observe the slowing and deepening of the respiratory rhythm. Sever the remaining vagus and note the intensification of the change. Stimulate the central end of one of the nerves with a tetanizing current of varying strength, and record the different effects produced. (Head, J. Physiol., 1889, x, pp. 1 and 279.)

Respiration in the Cat. — The experiment will be conducted by groups of four, designated as Operator, Assistant, Kymograph Manipulator, and Recorder. The cats when delivered have already been anaesthetized with urethane (2 gm. per kg.) given by stomach tube. Prepare for the experiment by performing the following operations; — tracheotomy and cannulation, cannulation of the right carotid, cannulation of the left femoral vein, exposure of the right and left vagi, and exposure of the right crural nerve.

Blood pressures may be taken entirely with the membrane manometer, but it must be calibrated with the mercury manometer at the end of the experiment. Respiration will be recorded by a light lever moved by a thread which passes over pulleys and is attached to hairs of the upper abdomen.

The Effect of Asphyxia. — Fill a small rubber bag with expired air and attach it to the tracheal cannula. Let the animal breathe the contents to and fro, while the record of blood pressure and respiration is being taken. The danger in this procedure is to the heart. Stop at once when the blood pressure begins to fall.

The Effect of Sensory Stimulation. — Stimulate the crural nerve centrally while recording respiration and blood pressure.

What do you conclude as to the influence on respiration of stimulating certain afferent fibres?

The Effect of Injecting Acid. — With blood pressure and respiration recording, introduce rapidly 10 c.c. of 1 per cent lactic acid into the femoral vein. Stop as soon as a positive effect is observed.

Is there any effect on respiration?

How do you explain the result?

Is the effect as great as that induced in asphyxia?

What are your conclusions as to the normal stimulus for the respiratory center?

Wash thoroughly with normal salt solution the burette used for injection.

The Effect of Vagotomy. — While recording respiration and blood pressure, cut both vagi.

What is the effect on rate and depth of respiration?

Was there any alteration on section of the first vagus nerve?

Why does respiration continue?

The Effect of Asphyxia after Vagotomy. — Recording as before, produce another short period of asphyxia.

What part did the vagi play in the previous period of asphyxia?

The Effect of Central Vagus Stimulation. — Stimulate the central end of either vagus with a strong tetanizing current while recording respiration and blood pressure.

Stimulate with weak single shocks.

Is there any difference in effect?

What do you conclude as to the function of the vagi in respiration?

Compare the results with the usual account given in textbooks. Urethane may modify the action of the vagi.

The Effect of an Irritant on the Nasal Mucosa. — Through a capillary pipette blow into a nostril two or three drops of ammonium hydrate.

What is the immediate effect on respiration?

What is the effect on blood pressure?

What are the secondary effects on respiration?

The Effect of Lack of Oxygen. — With the small hold in the tracheal cannula closed, connect with a T-tube conveying a stream of nitrogen. Record blood pressure and respiration as the animal dies.

How does the effect on respiration compare with that obtained in asphyxia?

What is the appearance of the blood?

What is the cause of death?

Do the effects so obtained correspond with those observed in illuminating gas poisoning? Explain the situation presented by both cases.

(Demonstration. — Respiration with the Thorax Open: Artificial Respiration. — Intratracheal Insufflation. — Anaesthetize a cat with ether, and fasten in the trachea a T-shaped cannula. Connect one limb of the cannula with a tube dipping about 6 cm. below the level of the water in a flask. Conduct from an air blast a stream of air through an ether bottle, or through a tube alone, into the other limb of the tracheal cannula. By this arrangement the animal is made to breathe air under a pressure slightly greater than 6 cm. of water. This pressure can be varied at will by changing the depth of the tube in the water. While the animal is thus breathing air at more than atmospheric pressure, open the chest by dividing the sternum along the middle line. Spread the ribs apart. The heart and lungs are thus exposed with very little loss of blood. Why do the lungs not collapse?

Raise the tube out of the water. What now happens to the lungs? Why?

Plunge the tube deep below the surface. What is the effect on the lungs? Explain. (Bauer u. Peterson, *Ztschr. f. physiol. Chem.*, 1904, xli, 299.)

Permit the animal to breathe ether in excess until respiration ceases and the heart is beating slowly. Stop the administration of ether and produce artificial respiration by rhythmically plunging the outlet tube under the water. Continue this process until the normal mechanism of respiration takes up its function.

Remove the tracheal cannula and replace it by a glass tube having about two-thirds the cross-section of the trachea. Let the tube, which is connected with the ether bottle and air blast, reach down the trachea to its bifurcation into the bronchi. Now admit the stream of air with sufficient force to keep the lungs moderately distended. Sever both phrenic nerves and determine whether the air blast alone can maintain contractions of the heart. (See Auer and Meltzer, *Proc. Soc. Exp. Biol. Med.*, 1909, VI, p. 107.)

Remove the collapsed lungs with the heart from the body and throw them into water. The lungs of newly born infants that have never breathed do not float. This is the "hydrostatic test.")

Artificial Respiration in Man

In some emergencies, such as electric shock and drowning, it is important to be able to cause artificial respiration. Various methods have been devised, but the essential feature of all of them is to reproduce as nearly as possible the normal ventilation of the lungs. It is of the first importance, of course, to make sure that the respiratory passages are free, and that the tongue does not in any way interfere with the movement of air through the pharynx. Employ the following methods of respiration and let the subject in each case suspend respiration and trust only to the artificial ventilation of his lungs. Report which method is found more effective.

The Silvester Method.—Place the subject on his back with the head at a lower level than the feet. Taking a position at the head of the subject, grasp his wrists, bring his forearms against the sides of his chest, press strongly downwards and inwards upon the ribs, and thus force air out of the lungs.

Release the pressure and permit the natural elasticity of the chest wall to restore its former size, and thus allow fresh air to enter the lungs. By extending the arms fully above the head, with a rapid pull at the end of the movement, the accessory muscles are drawn upon and the chest thus still further enlarged. Inspiration is now complete.

The first action is next repeated to cause a second expiration. Continue thus, at the rate of respiration already determined. In case of electric shock or drowning the process should be repeated until normal respiration is restored, or life is proved to be extinct. (Silvester, *The Discovery of the Physiological Method of Inducing Respiration in Cases of Apparent Death*, third ed., London, 1863.)

The Schäfer Method.—The advantages claimed for this method are that it assures a larger ventilation of the lungs, is less fatiguing to the operator, and is less likely to cause fracture of ribs than the other methods (Schäfer, *The relative efficiency of certain methods of performing artificial respiration in man*, Proc. Royal Soc., Edinburgh, 1904, xxv, 39).

Lay the subject on his belly, with one arm extended directly overhead, the other bent at the elbow and with the face to one side, resting on the hand or forearm, so that the nose and mouth are free for breathing.

Kneel straddling the subject's hips with your knees just below the patient's hip bones or opening of the trouser pockets. Place the palms of your hands on the small of his back with the fingers over the ribs, the little finger just touching the lowest rib, the thumb alongside of the fingers; the tips of the fingers just out of sight.

While counting one, two, and with arms held straight, swing forward slowly so that the weight of your body is gradually, but not violently, brought to bear upon the patient. This act should take from two to three seconds.

While counting three, swing backward so as to remove the pressure. The chest of its own elasticity will perform the function of inspiration.

While counting four, five — *rest*.

Repeat these operations, deliberately swinging forward and backward twelve to fifteen times a minute — a complete respiration in four or five seconds. Keep time with your own breathing. Continue until the issue is determined. In case of drowning it may be a half hour before respiration is restored. Schäfer's method is especially applicable to cases of drowning because the face is downwards and water in the air passages readily runs out.

*ANIMAL HEAT AND ITS REGULATION

THE bodily activities that have been studied — muscular contraction, the flow of blood, glandular activity, oxidations in the tissues — are all accompanied by the production of heat. All of the energy of the food that does not appear as mechanical work is given out by the body in the form of heat.

Heat is mainly produced as an accompaniment of muscular contraction. From the region in which it is produced it is distributed throughout the body by means of the blood stream. There is an optimum temperature for the most efficient exhibition of the bodily processes; in warm-blooded animals body temperature does not vary widely on either side of this optimum.

The Temperature of the Body

The Measurement of Body Temperature. — Apply a clinical thermometer for three minutes (1) under the tongue, (2) in the axilla with the arm closed upon it, and (3) in the rectum. Record the temperature in each region. Rectal temperature is less subject to variations from outside causes than either axillary or buccal temperature.

The Effects of Cold and Hot Drinks on Buccal Temperature. — Make observations on the temperature under the tongue before and after drinking very cold water. How much time is required for the return of the original temperature?

Repeat the observations before and after drinking water as hot as can comfortably be endured. How much time is required in this case for the return of the original temperature?

The Diurnal Variation of Body Temperature. — Record the buccal temperature (with proper precautions) on the even hours throughout twenty-four hours. Hand the results to the instructor, who will report to the class the average curve for all the observations. (See Jürgensen, *Die Körperwärme des gesunden Menschen*, Leipzig, 1873.)

The Difference between Warm- and Cold-Blooded Animals. — Find the temperature of a frog by placing a thermometer in its cesophagus. Keep the frog in water at 30° for 10 minutes. Again find the temperature of the animal. What is the change?

Put the animal now in cold water, and after 10 minutes observe its body temperature. What is the characteristic reaction of cold-blooded animals to external temperature variations?

Observe the rectal temperature of a guinea pig. Place the animal in a jar with a small opening in the cover, and set the jar in a tank of water heated to 35°. For 10 minutes observe the animal. How does the rate of respiration vary?

What is the effect of the observed change?

At the end of 10 minutes again take the temperature of the animal. What has been the effect of the warm surroundings on the body temperature?

Repeat the observations with the jar in water at 10° , and note the effect after 10 minutes.

What is the characteristic reaction of warm-blooded animals to variations in external temperature?

The Regulation of Body Temperature

In order to maintain the temperature at an optimum, mechanisms must exist for increasing the temperature when it tends to fall, and for lowering the temperature when it tends to rise.

The Mechanism for Heat Loss.—Run at top speed for 10 minutes, with the mouth closed. Immediately afterwards sit in a warm room and note any changes in the distribution of the blood as indicated by the color of the skin. Also note any appearance of sweat. What effects must the rapid respiration have on the loss of heat?

Has the muscular exercise caused any change in body temperature?

The Function of Sweating.—Place an equal amount of water in two beakers, and let them stand in a vessel containing boiling water. Cover the water in one beaker with oil, which prevents evaporation. In which beaker does the temperature of the water rise more rapidly? (Blagden, Phil. Trans. Royal Soc., London, 1775, lxxv, 111 and 484.)

(*Demonstration.*—*Nervous Stimulation of the Secretion of Sweat.*—A cat is etherized and anaesthesia maintained until death. The sciatic nerve is exposed and divided and the peripheral portion of the cut nerve stimulated with an interrupted current. Beads of sweat will appear on the pads of the feet. The sudorific nerves belong to the sympathetic system. (Goltz, Arch. f. d. ges. Physiol., 1875, xi, 71.))

The Mechanism of Heat Production.—Lie in cold water in a bath tub and observe the body temperature. Before there is any considerable loss of heat to the cold water shivering should occur and increase the heat supply. If for any reason this reflex regulation of heat production is impaired, body temperature may fall seriously. Such is the case in narcotization by alcohol and by the general anaesthetics.

(*Demonstration.*—*The Effect of Anaesthesia on Body Temperature.*—Observe the rectal temperature of a rabbit. Anaesthetize the animal with ether, chloral, or urethane. Lay on a table the animal with limbs extended, and note the rectal temperature during the course of an hour. What care should be taken of patients during prolonged operations? (Dumréil et Demarquay, Recherches expérimentales sur les modifications imprimées à la température animale par l'éther et par le chloroforme, Paris, 1848.))

J. G. L.

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